TESI DOCTORAL

Efectes de la D-cicloserina en la memòria i la plasticitat neural en animals sans i amb dèficit cognitiu

Marta Portero Tresserra

Tesi doctoral co-dirigida per:

Dra. Anna Vale Martínez   **Dra. Margarita Martí Nicolovius**

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Als meus pares

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ABREVIATURES

ABL	Amígdala basolateral
AMPA	Àcid α-amino-3-hidroxi-5-metil-4-isoxazol propiònic
AMPc	Adenosin monofosfat cíclic
ACh	Acetilcolina
APV	Àcid D,L-2-amino-5-fosfonovaleric
BDBv	Banda diagonal de broca, banda vertical
CA	*Cornus ammonis*
CIL	Còrtex infralímbic
CPF	Còrtex prefrontal
CPFm	Còrtex prefrontal medial
CPL	Còrtex prelímbic
CREB	Proteïna d'unió a l'element de resposta a l'AMP cíclic
CS$_2$	Disulfur de carboni
DCS	D-cicloserina
DLT	Depressió a llarg termini
DSO	Discriminació simple d'olors
EAAT	Transportadors d'aminoàcids excitadors
EC	Estímul condicionat
EI	Estímul incondicionat
ERK ½	Senyal extracel·lular reguladora de cinases 1 i 2
GABA	Àcid gamma-aminobutíric
GD	Gir dentat
Glu	Glutamat
HPC	Hipocamp
HPCd	Hipocamp dorsal

HPCv	Hipocamp ventral
LAM	Laberint aquàtic de Morris
MK-801	(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleat
NBM	Nucli basal magnocel·lular
NMDA	N-metil-D-aspartat
NMDAR1	Subunitat 1 del receptor NMDA
NMDAR2	Subunitat 2 del receptor NMDA
NMDAR2A	Subunitat 2A del receptor NMDA
NMDAR2B	Subunitat 2B del receptor NMDA
NMDAR3	Subunitat 3 del receptor NMDA
PB	Prosencèfal basal
PEP	Potencial excitador postsinàptic
PF	Nucli parafascicular
PLT	Potenciació a llarg termini
rAMPA	Receptors AMPA
RC	Resposta condicionada
rNMDA	Receptors NMDA
SCOP	Escopolamina
SI	Substància *innominata*
SM	Septe medial
TSPA	Transmissió social de preferència alimentària

I. Introducció

I. Introducció

L'objectiu general del treball de recerca que es presenta en aquesta tesi doctoral ha estat estudiar els efectes de l'administració de D-cicloserina (DCS), un agonista parcial dels receptors *N-m*etil *D-a*spartat (rNMDA), en la potenciació de la memòria i en la reversió dels dèficits cognitius induïts pel bloqueig de la transmissió colinèrgica i per l'envelliment natural. Concretament, hem analitzat els efectes de la infusió intracerebral de DCS en diferents regions cerebrals relacionades amb els processos d'aprenentatge i memòria com són l'amígdala basolateral (ABL), el còrtex prelímbic (CPL) i l'hipocamp (HPC), sobre la discriminació simple d'olors (DSO), la transmissió social de preferència alimentària (TSPA), el laberint aquàtic de Morris (LAM) i, finalment, sobre la potenciació a llarg termini (PLT). En aquest context, s'han realitzat quatre blocs experimentals: 1) avaluar els efectes de la DCS injectada a l'ABL sobre l'extinció i la reconsolidació de la DSO; 2) analitzar els efectes de la DCS al CPL en la reversió dels dèficits provocats per l'administració d'escopolamina (SCOP) en la consolidació de la memòria de la DSO i la TSPA; 3) estudiar els efectes de la DCS administrada a l'HPC en la compensació del deteriorament de la TSPA i de la plasticitat sinàptica causat per la infusió d'SCOP; 4) avaluar els efectes de la DCS a l'HPC sobre l'adquisició i el record de la TSPA i el LAM en animals vells.

Aquesta tesi doctoral s'emmarca dins dels projectes d'investigació titulats "Implicació dels sistemes de neurotransmissió colinèrgic i glutamatèrgic en la modulació dels processos cognitius: atenció, aprenentatge i memòria (PSI2008-06267) i "Potenciació de l'atenció i de la memòria en models animals de dèficit cognitiu per envelliment i lesió cerebral" (PSI2011-26862) dirigits per la Dra. Margarita Martí Nicolovius i la Dra. Gemma Guillazo Blanch, els quals formen part d'una de les línies de recerca del laboratori de Psicobiologia de l'Institut de Neurociències de la Universitat Autònoma de Barcelona.

Els treballs d'aquesta tesi doctoral es presenten en forma de compendi de publicacions. El primer article porta per nom "*D-cycloserine in the basolateral amygdala prevents extinction and enhances reconsolidation of odor-reward associative learning in rats*" i ha estat publicat a la revista *Neurobiology of Learning and Memory* (2013) 100: 1-11. Aquest estudi avalua els efectes de la injecció de DCS abans de l'extinció i la reconsolidació de la DSO. Els resultats mostren que la DCS impedeix l'aprenentatge i la retenció de l'extinció (experiment 1) i afavoreix la reconsolidació de la memòria (experiment 2). El segon article, que porta per títol "*D-cycloserine in prelimbic cortex reverses scopolamine-induced deficits in olfactory memory in rats*", ha estat publicat a la revista PLoS

ONE 8(8): e70584. doi:10.1371/journal.pone.0070584, i té com a objectiu principal avaluar la capacitat de la DCS al CPL per revertir els dèficits de memòria induïts per l'administració d'SCOP. Els resultats d'aquest treball posen de manifest que la DCS permet potenciar i compensar les alteracions en la DSO, reverteix aquest deteriorament en la TSPA quan s'avalua amb un test de memòria amb dues opcions de resposta (experiment 3) i pal·lia completament el dèficit cognitiu quan es mesura en un test de memòria amb tres opcions de resposta (experiment 4).

El tercer manuscrit, que porta per títol "*D-cycloserine prevents relational memory deficits and suppression of long-term potentiation induced by scopolamine in the hippocampus*", ha estat enviat a la revista *Hippocampus* (2013) per a la seva publicació i té com a objectiu principal analitzar l'efecte de la DCS a l'HPC ventral (HPCv) per contrarestar els impediments en la memòria de la TSPA provocats per l'SCOP (experiment 5), així com avaluar els efectes de la DCS sobre la plasticitat sinàptica hipocampal (experiment 6). Aquestes investigacions suggereixen que la DCS permet pal·liar els problemes en la memòria relacional així com en el manteniment de la PLT derivades del bloqueig colinèrgic ocasionat per l'SCOP.

Finalment, el quart manuscrit, anomenat "*D-cycloserine reverses hippocampal-dependent memory deficits in aged rats*", s'enviarà a la revista *Neurobiology of Aging* (2013) per a la seva publicació. En aquest estudi (experiment 7) hem determinat la capacitat de la DCS a l'HPCv per poder disminuir el deteriorament cognitiu associat a l'envelliment en dues tasques relacionals hipocamp dependents, una de memòria olfactòria com és la TSPA i una de memòria espacial com és el LAM. Els resultats corroboren que durant l'envelliment es produeix una afectació de l'aprenentatge i la memòria i demostren que l'administració de DCS permet revertir els dèficits en la consolidació de la memòria de la TSPA i en l'aprenentatge de reversió en el LAM.

II. Plantejament i Objectius

II. Plantejament i Objectius

El progressiu envelliment de la població comporta un increment de la incidència de malalties neurodegeneratives que es manifesten amb deteriorament cognitiu i constitueix un dels principals problemes de salut de les societats modernes. El desenvolupament de tractaments farmacològics capaços de mitigar aquests problemes suposa un repte per a la Neurociència actual i ha situat els investigadors davant la necessitat de comprendre els mecanismes neurals subjacents a la formació de la memòria i la seva potenciació. En aquest context s'emmarquen els experiments realitzats en el present treball de tesi doctoral que té com a objectiu principal investigar els possibles efectes beneficiosos de la DCS, un fàrmac que potencia la transmissió glutamatèrgica i que en els darrers anys s'ha reconegut com a un possible potenciador cognitiu. En els experiments que hem realitzat, la DCS s'ha administrat a diferents regions cerebrals de la rata, com l'ABL, l'HPCv i el CPL, les quals formen part dels circuits neuroanatòmics que participen en l'aprenentatge i la memòria de diverses tasques de tipus olfactori i espacial. També s'ha estudiat la capacitat d'aquest fàrmac per a reduir els dèficits cognitius resultat de la hipofunció colinèrgica i l'envelliment normal, així com els possibles mecanismes d'acció de la DCS com a potenciador cognitiu a nivell cel·lular.

El Glutamat (Glu) és el principal neurotransmissor excitador del sistema nerviós central (Beart & O'Shea 2007) i les sinapsis glutamatèrgiques tenen un paper fonamental en les funcions cognitives, especialment els rNMDA i els receptors α-amino-3-hidroxi-5-metil-4-isoxazolpropionic (rAMPA), ja que participen molt directament en la plasticitat sinàptica necessària per l'aprenentatge i la formació de la memòria (Abraham & Bear 1996; Bliss & Collingridge 1993). El rNMDA, en concret, presenta unes característiques que el converteixen en una diana clau pel disseny de possibles tractaments farmacològics, ja que conté un canal que permet el pas d'ions de Ca^{2+} a l'interior de la neurona postsinàptica, els quals indueixen canvis, com són la fosforilació dels rAMPA, les modificacions del citoesquelet que alteren l'estructura de les espines dendrítiques o bé l'activació de factors de transcripció que permeten la síntesi de noves proteïnes. És important destacar que tots aquests canvis promouen un augment de l'eficiència de les sinapsis (Morgado 2011; Xia & Storm 2005). En consonància amb l'anterior, s'ha demostrat que el bloqueig d'aquests receptors ionotròpics modifica la transmissió glutamatèrgica i provoca una disrupció de la inducció i/o el manteniment de la PLT, un dels principals mecanismes cel·lulars de plasticitat sinàptica que estan involucrats en la formació de la memòria (Granger et al. 2013). No obstant, malgrat que la potenciació de l'activitat glutamatèrgica podria ser un mecanisme adequat per a millorar els processos cognitius (Collingridge 2013), un excés d'activitat podria promoure processos de mort

cel·lular, fet que fa necessari aconseguir fàrmacs capaços d'activar els receptors glutamatèrgics evitant els possibles efectes de neurotoxicitat. En aquest sentit, una via eficient per a millorar els processos cognitius (Jansen & Dannhardt 2003) i potenciar la plasticitat sinàptica (Potier et al. 2010; Abe et al. 1990) podria consistir en la modulació del lloc d'unió de l'aminoàcid coagonista glicina en el rNMDA, el qual implica un menor risc de neurotoxicitat en comparació amb una modulació directa del receptor (Barth et al. 2005).

Estudis previs han demostrat que la DCS, agonista del lloc d'unió de la glicina del rNMDA (Normann 2008), és capaç de facilitar l'adquisició i la consolidació de la memòria de diferents paradigmes d'aprenentatge associatiu (Bustos et al. 2010; Curlik & Shors 2011; Davis 2002; Ledgerwood et al. 2005; Lee et al. 2006; Rodgers et al. 2011; Villarejo-Rodriguez et al. 2010). No obstant això, un gran nombre d'antecedents s'ha centrat en els efectes de la DCS en els processos d'extinció i reconsolidació, ja que la seva modulació pot ser utilitzada en el tractament de conductes no adaptatives i els trastorns d'ansietat en els éssers humans (Davis et al. 2006; Hofmann 2013). Encara que ambdós processos es desencadenen a partir de la recuperació de la memòria, durant la reconsolidació la traça de memòria es torna làbil i, per tant, pot ser enfortida o modificada, mentre que l'extinció debilita la memòria original, possiblement a través de la formació d'un nou aprenentatge (de la Fuente et al. 2011). L'administració sistèmica de DCS abans i després de l'adquisició de l'extinció facilita el seu aprenentatge en tasques de tipus aversiu, en especial el condicionament de la resposta de por (Ledgerwood et al. 2005). En relació amb l'extinció d'aprenentatges de tipus apetitiu, la majoria d'estudis ha utilitzat paradigmes d'addicció a drogues i els resultats obtinguts han generat certa controvèrsia. Quan la DCS s'administra després de l'aprenentatge d'extinció es sol millorar la retenció mentre que quan s'administra abans alguns estudis obtenen facilitació del condicionament inicial i d'altres no troben cap efecte (Thanos et al. 2011; Lu et al. 2011). Un menor nombre d'estudis ha tractat tasques apetitives que utilitzen reforçadors naturals, tals com el menjar, i troben resultats inconsistents (Flavell et al. 2011) que podrien dependre del moment en què s'administra el fàrmac (Port & Seybold 1998; Vurbic et al. 2011). En humans, l'administració de DCS abans de l'extinció no sembla exercir cap efecte i, fins i tot, pot produir un enfortiment de l'aprenentatge previ (Price et al. 2012). Un menor nombre d'estudis ha considerat el procés de reconsolidació i en tots ells l'administració de DCS preentrenament va facilitar aquest procés, tant en tasques de tipus aversiu com apetitiu (Bustos et al. 2010).

L'ABL és la regió cerebral més estudiada en relació amb l'extinció i la reconsolidació de la memòria (Quirk & Mueller 2008; Tronson & Taylor 2007). S'ha observat que les injeccions de DCS directament a l'ABL de rates produeixen efectes facilitadors en ambdós processos, generalment en

tasques aversives (Rehberg et al. 2010). No obstant això, existeix una menor quantia de treballs que avaluï els efectes de la DCS administrada intra-ABL en paradigmes apetitius, i els únics estudis existents valoren l'administració postextinció i obtenen resultats divergents (Torregrossa et al. 2010; Botreau et al. 2006). Per tant, partint de la hipòtesi que la potenciació de la transmissió glutamatèrgica a l'ABL modula l'extinció i la reconsolidació de la memòria, els primers experiments del present treball van tenir com a objectiu verificar si la DCS injectada en aquest nucli amigdalí podria millorar l'extinció i/o la reconsolidació d'una tasca associativa de naturalesa olfactòria, la DSO. La DSO consisteix en un aprenentatge apetitiu de ràpida adquisició en el qual l'animal ha d'aprendre a discriminar entre diferents estímuls olfactoris diferents i associar-ne un al reforç positiu (Sara et al. 1999). Aquesta tasca sembla dependre especialment del CPL i de l'ABL, i és sensible tant a la modulació glutamatèrgica com a la colinèrgica. Concretament, els estudis previs indiquen que l'administració de DCS al CPL permet potenciar la retenció d'aquesta tasca (Villarejo-Rodriguez et al. 2010), mentre que el bloqueig colinèrgic provoca una disrupció en la consolidació de la mateixa (Carballo-Marquez et al. 2009a).

La DCS també s'ha mostrat com un fàrmac capaç de millorar el deteriorament cognitiu associat a manipulacions cerebrals i conductuals. Per exemple, l'administració sistèmica de DCS redueix els dèficits deguts a la privació de son (Silvestri & Root 2008), a l'estrès (Yamamoto et al. 2008), a lesions cerebrals (Land & Riccio 1999; Kawabe et al. 1998; Puumala et al. 1998; Waddell et al. 2009), a l'envelliment (Baxter et al. 1994; Thompson & Disterhoft 1997) o bé induïts per diverses manipulacions farmacològiques (Fishkin et al. 1993; Ohno & Watanabe 1996; Andersen et al. 2002). En el present treball ens vam plantejar que el bloqueig de l'activitat colinèrgica podria causar dèficits mnemònics, els quals serien susceptibles de ser revertits mitjançant l'administració de DCS. Per a tal plantejament ens vam basar en diversos antecedents. En primer lloc, l'acetilcolina (ACh) és un dels neurotransmissors que més s'ha relacionat amb els processos d'aprenentatge i memòria (Hasselmo 2006) i, a més, s'observa una important pèrdua de cèl·lules colinèrgiques que correlaciona amb les alteracions de memòria que apareixen durant el transcurs de la malaltia d'Alzheimer (Whitehouse et al. 1982). Addicionalment, els fenòmens de plasticitat cerebral subjacents als processos d'aprenentatge i memòria, com la PLT, poden ser regulats mitjançant la modulació dels receptors colinèrgics (Markram & Segal 1990). Un dels models farmacològics d'alteració de la neurotransmissió colinèrgica més estudiats ha estat l'administració d'SCOP (Kilnkenberg & Blockland 2010). S'ha demostrat que aquest antagonista dels receptors muscarínics impedeix l'adquisició de nova informació i interromp el procés de consolidació de la memòria declarativa en humans (Petersen 1977). En animals, les infusions d'SCOP en diferents regions cerebrals alteren l'execució de paradigmes que avaluen la memòria de treball (Mishima et al. 2000), la memòria espacial (Nieto-

Escamez et al. 2002), l'atenció (Chudasama et al. 2004) i tasques d'aprenentatge de condicionament clàssic i instrumental (Passani et al. 2001; See et al. 2003). De forma complementària, estudis de plasticitat cerebral demostren que l'administració d'SCOP provoca supressió de la PLT en experiments *in vitro* i *in vivo* (Hirotsu et al. 2003; Ovespian et al. 2004).

D'especial interès pel nostre treball és el fet que els sistemes glutamatèrgic i colinèrgic podrien interactuar en la formació de la memòria. En aquest sentit, estudis conductuals que avaluen els efectes de manipulacions farmacològiques mostren que els antagonistes dels rNMDA impedeixen els efectes facilitadors produïts per l'administració de fisostigmina, un inhibidor de la degradació de l'ACh, mentre que l'administració conjunta de dosis inefectives d'NMDA i fisostigmina faciliten la consolidació de la memòria (Jafari-Sabet 2006). A més, l'administració sistèmica o a l'HPC de dosis subllindar d'antagonistes dels rNMDA juntament amb antagonistes dels receptors muscarínics deteriora l'aprenentatge de diverses tasques (Figueredo et al. 2008; Khakpai et al. 2012). En congruència amb l'exposat anteriorment, s'ha descrit que el deteriorament cognitiu produït per l'administració sistèmica d'SCOP podria ser revertit mitjançant l'administració també sistèmica de DCS (Andersen et al. 2002). En relació amb l'administració intracerebral d'aquest fàrmac, s'ha observat que la DCS injectada intra-HPC abans de l'entrenament és capaç de pal·liar els dèficits de memòria de treball produïts pel bloqueig muscarínic (Ohno & Watanabe 1996).

Així doncs, d'acord amb la hipòtesi de què els sistemes glutamatèrgics i colinèrgics interactuen d'una forma sinèrgica o complementària al CPL i a l'HPC en la modulació de l'aprenentatge i la memòria, vam dissenyar una sèrie d'experiments en els quals vam injectar SCOP intracerebralment i vam valorar la capacitat de la DCS per reduir els possibles dèficits cognitius derivats de la depleció colinèrgica. En el primer estudi vam examinar la capacitat de la DCS administrada directament al CPL per disminuir els dèficits de memòria produïts per l'SCOP en dues tasques apetitives de naturalesa olfactòria: la DSO, tasca de tipus olfactori que hem descrit anteriorment, i la TSPA. La TSPA, a diferència de la DSO, requereix un tipus d'aprenentatge associatiu més complex com és l'aprenentatge relacional. Aquest aprenentatge es realitza a través de la interacció social entre individus de la mateixa espècie, durant la qual l'animal observador associa l'aroma de l'aliment que l'animal demostrador ha ingerit amb un component del seu alè, i després recorda aquesta associació en un test de preferència alimentària (Galef & Wigmore 1983). Diversos estudis han demostrat que la TSPA és una tasca fonamentalment hipocamp dependent (Winocur et al. 2001), però també requereix la integritat del còrtex prefrontal (CPF), en especial el CPL (Smith et al. 2007). Aquesta estructura cortical està relacionada específicament amb la flexibilitat cognitiva i la inhibició conductual i sembla, doncs, que la seva implicació augmenta quan la TSPA comporta diferents opcions de resposta (Winocur et al. 1999).

En el tercer grup d'experiments vam continuar estudiant la hipòtesi de la interacció dels sistemes colinèrgic i glutamatèrgic en la modulació de la memòria, però en una altra àrea cerebral, l'HPC, en la qual també vam analitzar efectes cel·lulars. Es va realitzar un primer treball en el qual vam determinar els efectes de l'administració de DCS i d'SCOP a l'HPCv en la TSPA. Tal com hem indicat, aquesta tasca de memòria relacional és hipocamp-dependent i requereix una correcta transmissió muscarínica en la regió ventral de l'HPC, tal com suggereix un estudi precedent que mostra que l'administració d'SCOP en aquesta regió impedeix el record de la tasca (Carballo-Marquez 2009a). En el segon treball, vam estudiar els efectes de la DCS a nivell cel·lular, realitzant un experiment *in vitro* que investigava els efectes de la DCS i l'SCOP sobre la PLT en seccions hipocampals. Estudis previs havien demostrat que la infusió de DCS amplificava els potencials excitadors postsinàptics (PEP), facilitava el manteniment de la PLT (Rouaud & Billard 2003) i revertia el deteriorament que s'observa en diferents models de dèficit cognitiu (Kochlamazashvili et al. 2012; Yaka et al. 2007; Billard & Rouaud 2007). Aquests antecedents semblen suggerir que un dels substrats neurobiològics on podria actuar la DCS per tal de produir efectes de millora cognitiva seria enfortint la força de les connexions sinàptiques. No obstant això, no hem localitzat estudis que avaluïn els efectes de la perfusió de DCS per revertir els dèficits en la plasticitat sinàptica causats per un bloqueig colinèrgic. Així doncs, en aquest experiment vam analitzar els efectes de la infusió de DCS i SCOP després de l'estimulació d'alta freqüència en les col·laterals de Schaffer per tal de generar PLT a la regió CA1 hipocampal.

En l'últim treball ens vam centrar en la capacitat de la DCS per compensar els dèficits cognitius associats a l'envelliment normal. Durant el procés fisiològic d'envelliment s'ha observat un declivi de les funcions cerebrals que comporta una pèrdua progressiva de les capacitats cognitives, les quals inclouen dèficits d'aprenentatge i memòria (Yankner et al. 2008). Diversos estudis indiquen que l'HPC, el qual, com ja hem esmentat, és crític per a l'aprenentatge relacional (Driscoll & Sutherland 2005), és una estructura que pateix canvis estructurals i fisiològics durant l'envelliment que podrien explicar el substrat dels dèficits cognitius. Un dels factors que pot contribuir al deteriorament observat en models animals d'envelliment és la reducció en el nombre de receptors glutamatèrgics NMDA i AMPA a l'HPC (Rosenzweig et al. 2003), els quals són fonamentals per a l'expressió de la plasticitat sinàptica implicada en l'aprenentatge i la memòria (Martin et al. 2000). Per aquest motiu, en els darrers anys s'ha investigat la capacitat de la DCS per a revertir els dèficits cognitius associats a l'edat. Si bé alguns antecedents han mostrat que la DCS administrada per via sistèmica reverteix els dèficits cognitius, especialment en tasques hipocamp dependents (Baxter et al. 1994; Riekkinen & Riekkinen 1997a; Aura et al. 1998), no existeixen estudis que investiguin les

regions cerebrals que podrien estar implicades en aquesta potenciació. Així, sota la hipòtesi de què la potenciació de la transmissió glutamatèrgica a l'HPC podria modular positivament el deteriorament cognitiu degut a l'envelliment, en el present estudi vam avaluar els efectes de l'administració de DCS directament a l'HPCv de rates velles en dues tasques hipocamp dependents, una de tipus olfactori com és la TSPA i una altra espacial, com és el laberint aquàtic de Morris. Cal remarcar que la recerca sobre la memòria olfactòria podria ser especialment adient, ja que s'ha demostrat que un deteriorament en la identificació, el reconeixement i el llindar de detecció de les olors està present en l'envelliment i en algunes malalties neurodegeneratives (Bahuleyan & Singh 2012). D'altra banda, el LAM, és un paradigma d'aprenentatge espacial en el qual els animals han de trobar una plataforma submergida guiant-se per unes pistes contextuals externes. Aquesta tasca depèn de la integritat de l'HPC i els seus rNMDA, fet que ha estat evidenciat en experiments que utilitzen lesions hipocampals o antagonistes dels rNMDA i observen dèficits en el seu aprenentatge i record (Terry 2009; Morris et al. 1986). Així doncs, tenint en compte que durant l'envelliment s'afecta especialment l'aprenentatge espacial (Driscoll & Sutherland 2005) i que aquest dèficit podria ser compensat amb algun tractament farmacològic com la DCS, en aquest darrer experiment ens vam plantejar l'administració a l'HPCv d'aquest compost per tal d'intentar revertir els possibles dèficits observats en el LAM, a més de la TSPA.

En resum, en aquest treball de tesi doctoral hem estudiat la capacitat de la DCS administrada en diverses regions cerebrals com l'ABL, el CPL i l'HPCv per facilitar els processos d'extinció i reconsolidació, per reduir els dèficits de memòria produïts per la hipofunció colinèrgica i l'envelliment normal, així com per modular la plasticitat neural subjacent als processos d'aprenentatge i memòria. Si bé ambdós models de dèficit comporten dificultats cognitives, el primer reproduiria, tot i que de forma temporal i reversible, una situació patològica similar a la que es podria observar en malalties neurodegeneratives, mentre que el segon imitaria els dèficits cognitius de tipus més heterogeni que apareixen en persones d'avançada edat.

Així doncs, els objectius específics plantejats en aquesta tesi doctoral han estat els següents:

1. Verificar si la DCS, agonista parcial dels rNMDA, injectada a l'ABL pot millorar l'extinció i/o la reconsolidació d'una tasca associativa de naturalesa olfactòria, la DSO.

2. Estudiar si la DCS injectada al CPL pot revertir els dèficits de memòria en la DSO induïts pel bloqueig de receptors muscarínics amb l'administració d'SCOP.

3. Determinar si la infusió de DCS al CPL pot alleujar el deteriorament de la memòria relacional, avaluada en la TSPA, provocat per la injecció d'SCOP.

4. Analitzar si la DCS administrada a l'HPCv pot contrarestar els dèficits mnemònics en la TSPA causats per la infusió d'SCOP.

5. Investigar si la perfusió de DCS en seccions hipocampals pot compensar l'alteració en la PLT promoguda per la perfusió d'SCOP.

6. Avaluar si la DCS injectada a l'HPCv pot disminuir la pèrdua de memòria relacional, tant de naturalesa espacial com no espacial, produïda per l'envelliment normal en la TSPA i el LAM.

III. Marc Teòric i Antecedents Experimentals

III. Marc teòric i antecedents experimentals

## 1.	Modulació glutamatèrgica de l'aprenentatge i la memòria

### 1.1	El glutamat

El Glu representa el principal neurotransmissor excitador del sistema nerviós central (Beart & O'Shea 2007; Marmiroli & Cavaletti 2012; Molinari et al. 2012), és el responsable del 75% de la transmissió excitadora de l'encèfal i és un aminoàcid fonamental en el metabolisme energètic i en la síntesi de noves proteïnes (Mattson 1996) (Figura 1). Degut a aquests i d'altres factors, les sinapsis glutamatèrgiques estan implicades en processos de formació de la memòria i de plasticitat neural (Abraham & Bear 1996; Collingridge & Bliss 1995; Riedel 1996). A més a més, els canvis en la transmissió glutamatèrgica s'han relacionat amb condicions neuropatològiques com l'epilèpsia, la isquèmia cerebral, l'esclerosi lateral amiotròfica, la malaltia d'Alzheimer, la malaltia de Parkinson i l'esquizofrènia (Danbolt 2001).
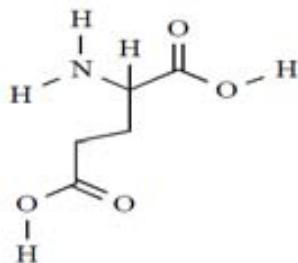
Figura 1. Estructura química del glutamat

Pel que fa a la seva síntesi al sistema nerviós, la glucosa constitueix el principal precursor i accedeix al sistema nerviós central travessant la barrera hematoencefàlica mitjançant transportadors específics (Prebil et al. 2011; Devraj et al. 2011). A l'interior dels astròcits, la glucosa es transforma en lactat que és captat per les neurones i transformat en acetil-coenzima A. Aquest ingressa en el cicle de Krebs, on es transforma en α-cetoglutarat, el qual constitueix el substrat principal per la seva transaminació a Glu. D'altra banda, el Glu neuronal té altres orígens, com la glutamina, que es sintetitzada per les cèl·lules glials des d'on es transporta a les terminacions nervioses per convertir-se en Glu mitjançant l'acció de la glutaminasa, un enzim mitocondrial (Hertz et al. 1999) (Figura 2).
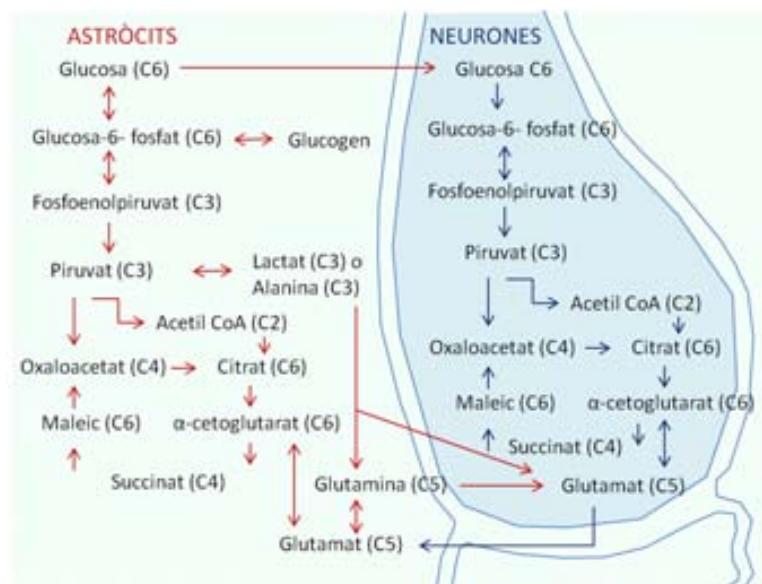
Figura 2. Interaccions metabòliques entre astròcits i neurones en la síntesi del glutamat sinàptic (Adaptat d'Hertz et al. 1999).

El Glu s'emmagatzema en vesícules al terminal sinàptic i és alliberat quan un impuls elèctric activa els canals de Ca^{2+} dependents de voltatge. Finalment, és eliminat de l'espai sinàptic per tal d'evitar processos de neurotoxicitat (Boston-Howes et al. 2006; Camacho & Massieu 2006) i pot ser recaptat pels astròcits o per les neurones.
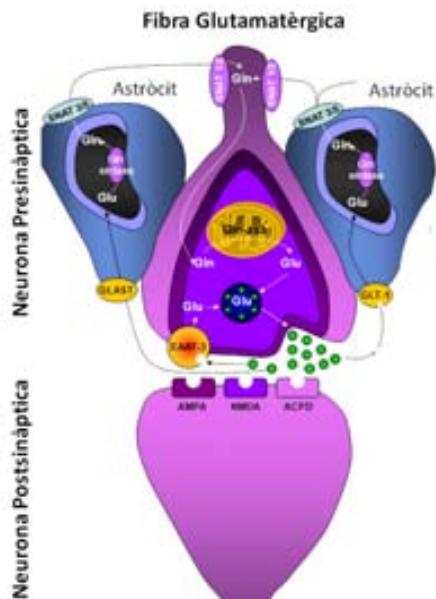
En el primer cas, els transportadors GLT-1 i Glast porten el Glu a la cèl·lula glial on es transforma en glutamina per acció de la glutamina transferasa i, a continuació, queda emmagatzemat com a reserva als mitocondris de la neurona. En el segon cas, el Glu és recaptat pels transportadors EAAT per accedir a l'interior mitocondrial on és transformat en α-cetoglutarat (Figura 3). El cicle de síntesi i degradació del Glu requereix elevades quantitats d'energia i es calcula que el cicle complet consumeix el 3% del total d'energia obtinguda pel metabolisme de la glucosa (García-Espinosa et al. 2004).

Figura 3. Esquema del metabolisme neuronal i glial del glutamat

1.2 Receptors del glutamat

Existeixen dos tipus de receptors del Glu: els ionotròpics i els metabotròpics. Els receptors ionotròpics estan associats a canals iònics que desencadenen fenòmens elèctrics d'activació ràpida i fins al moment se n'han caracteritzat tres tipus: els rNMDA, els rAMPA i els receptors cainat. S'ha estimat que aproximadament un 70% de les sinapsis del sistema nerviós contenen rNMDA o AMPA (Bekkers & Stevens 1989), amb una major densitat a l'escorça cerebral, la formació hipocampal, l'amígdala i l'estriat.

El rNMDA és el receptor del Glu més estudiat i s'ha descrit que conté sis llocs d'unió pel neurotransmissor: quatre localitzats a la part externa del receptor i dos localitzats dins del canal iònic. L'obertura del canal del rNMDA requereix, a part del Glu, la presència d'un coagonista, l'aminoàcid glicina (Johnson & Ascher 1987) (Figura 4) i que l'ió de Mg^{2+} deixi de bloquejar-lo. Per tant, és necessari que la membrana postsinàptica es trobi parcialment despolaritzada, principalment per l'activació dels rAMPA o cainat, per a que el Mg^{2+} pugui sortir i permetre el corrent iònic. Per tant, es podria considerar que el rNMDA és un canal iònic depenent de neurotransmissor i de voltatge. L'obertura del canal iònic controlat pel rNMDA permet l'entrada d'ions de Na^+ i de Ca^{2+} a la cèl·lula i la sortida de K^+, els quals produeixen la despolarització de la neurona postsinàptica. L'entrada de Ca^{2+} actua com a segon missatger per activar cascades de senyalització intracel·lular, com cinases i fosfatases, les quals alteren les característiques de les sinapsis.
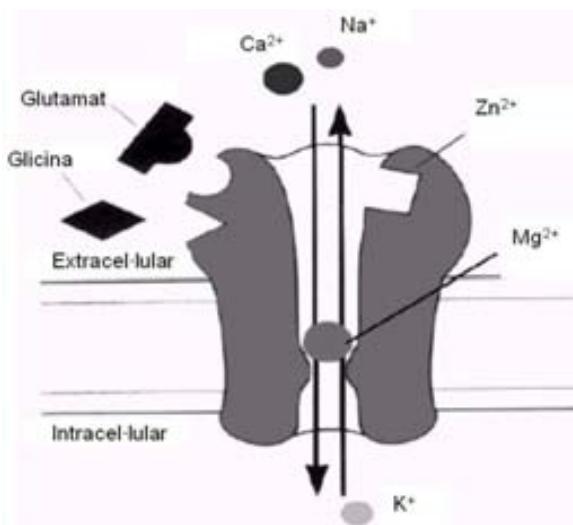
Figura 4. Representació gràfica del receptor NMDA. Conté el lloc d'unió pel Glu i el coactivador glicina i també un lloc d'unió pel Mg^{2+} en el porus del canal.

El canal del rNMDA és un heterotetràmer compost per diferents combinacions de vàries subunitats (denominades NMDAR1, NMDAR2 i NMDAR3). A cada sinapsi, en funció d'aquestes

subunitats, es produiran efectes postsinàptics diferents en resposta al Glu. Per exemple, dins dels rNMDAR2, els rNMDA2A i rNMDA2B estan relacionats amb la plasticitat sinàptica depenent dels rNMDA i estan distribuïts per tot el cervell mentre que el rNMDA2C i el rNMDA2D tenen localitzacions més específiques, com són el cerebel i el tàlem respectivament (Buller et al. 1994; Paoletti & Neyton 2007). El rNMDA es localitza bàsicament a la neurona postsinàptica però s'ha caracteritzat la seva presència també a la regió presinàptica (Rodriguez-Moreno et al. 2011), on sembla que té una funció moduladora de l'excitotoxicitat del Glu, augmentant i potenciant la resposta (Collingridge 1995). A causa de la seva multifuncionalitat i el seu paper fonamental en la formació de la memòria s'ha considerat una diana interessant per a la potenciació de les funcions cognitives (Collingridge et al. 2013; Lee & Silva 2009).

A més dels rNMDA, en els quals ens centrarem especialment en aquesta tesi doctoral, el Glu s'uneix a altres receptors com els AMPA, els quals regulen el potencial postsinàptic excitador d'acció ràpida després de l'alliberació del neurotransmissor. Com s'ha mencionat anteriorment, aquest canvi de polarització de la membrana és crucial per a l'activitat del rNMDA, ja que permet moure l'ió de Mg^{2+} que bloqueja el canal i així fer possible el trànsit d'ions. Les subunitats que el constitueixen estan codificades per la família de gens de receptors ionotròpics R1 - R4 i en funció de les seves composicions moleculars, el canal iònic que porta associat pot ser permeable al Na^+, K^+, o fins i tot al Ca^{2+}. Així, els receptors GluR1, GluR3 i GluR4 són permeables al Ca^{2+}, mentre que la subunitat GluR2 no ho és (Geiger et al. 1995; Jonas & Burnashev 1995). El receptor cainat també forma part dels receptors ionotròpics i s'activa com a conseqüència de la seva estimulació per cainat o Glu i, en menor mesura, per AMPA o NMDA. Depenent de la composició de les subunitats que el conformen, el canal associat pot ser permeable al Ca^{2+} o a d'altres cations (Paternain et al. 1998).

D'altra banda, els receptors metabotròpics del Glu són receptors associats a proteïnes G i quan s'estimulen es promou o inhibeix l'activació de cascades metabòliques intracel·lulars en el citoplasma, en la membrana i inclús en el nucli cel·lular (Krieger et al. 2000). Les accions metabotròpiques es caracteritzen per ser perllongades en el temps i es poden traduir en adaptacions moleculars i canvis en l'expressió gènica (Di liberto et al. 2010). Aquesta circumstància és vital per als mecanismes moleculars subjacents a la plasticitat sinàptica i als processos de memòria.

1.3 Sistema glutamatèrgic, plasticitat neural i mecanismes d'aprenentatge i memòria

En els darrers anys, s'ha demostrat que la neurotransmissió glutamatèrgica, mitjançant la interacció amb receptors ionotròpics de tipus NMDA i AMPA i amb receptors metabotròpics mGluR1 i mGluR5, és un element fonamental per a la modificació de les connexions sinàptiques i pels processos de plasticitat cerebral (Marmiroli & Cavaletti 2012). En aquest sentit, s'ha observat que la densitat de rNMDA i AMPA és especialment rellevant per a la regulació de la plasticitat sinàptica i, per tant, per a les capacitats cognitives necessàries pels processos d'aprenentatge i memòria (Clayton et al. 2002).

De tots els canvis plàstics considerats com el substrat fisiològic que podria explicar la formació de la memòria, en el present treball ens centrarem en els mecanismes electrofisiològics associats a la plasticitat sinàptica modulada pels rNMDA i específicament en la PLT (Collingridge et al. 1988; Lynch 2004; Malinow & Malenka 2002; Granger et al. 2013). La PLT, descoberta per Terje Lømo (1966) i Tim Bliss (1973), consisteix en un canvi de llarga durada en l'eficàcia de la transmissió sinàptica que depèn de l'experiència. Aquests investigadors van observar que l'estimulació d'alta freqüència de la via perforant de l'HPC produïa un augment de l'amplitud dels PEP de les cèl·lules granulars del gir dentat que podia durar hores. És a dir, un augment de l'eficàcia de la transmissió sinàptica d'aquesta via (Bliss & Lømo 1973) (Figura 5). Des d'aquell moment, la PLT s'ha considerat una forma de plasticitat sinàptica que indueix mecanismes cel·lulars similars als que s'esdevenen durant la formació de la memòria.

Els canvis moleculars i estructurals involucrats en la PLT han estat àmpliament estudiats i impliquen modificacions postsinàptiques, com alteracions de l'estructura de les sinapsis i en la citoarquitectura de la mateixa neurona (Nguyen & Kandel 1997), i també canvis presinàptics, com ara l'alliberació del neurotransmissor. Actualment, la majoria d'experiments que estudien aquest mecanisme electrofisiològic es realitzen a les sinapsis entre les col·laterals de Schaffer i les neurones piramidals de l'àrea CA1 de l'HPC (Lüscher & Malenka 2012). Així, si els axons s'estimulen 3 o 4 vegades per minut l'amplitud dels PEP enregistrats en les neurones de CA1 es manté constant, i genera el que es denomina una PLT primerenca o *early*-LTP. No obstant això, una successió breu i d'alta freqüència d'estímuls, anomenada estimulació tetànica, en els mateixos axons indueix un augment perllongat de l'amplitud dels PEP, coneguda com PLT tardana o *late*-LTP. Durant la generació de PLT, l'augment en l'alliberació de Glu i l'activació dels rAMPA afavoreixen l'entrada de Ca^{2+} a la neurona postsinàptica a través dels rNMDA, fet que permet la facilitació persistent de la transmissió sinàptica i l'activació de diferents enzims, entre els quals es troben la proteïna cinasa C i la proteïna cinasa Ca^{2+}-calmodulina depenent de tipus II. Aquests enzims, activen diferents factors de

transcripció, com el factor d'unió a elements de resposta a l'AMPc (CREB), el qual indueix l'activació de certs gens que modifiquen l'estructura de la neurona postsinàptica i augmenten l'expressió de receptors. Totes aquestes modificacions permeten enfortir les connexions sinàptiques, de manera que davant d'un mateix estímul disminueix el llindar que desencadena una resposta. Aquest mecanisme de plasticitat sinàptica pot explicar molts tipus d'aprenentatges, des del relativament senzill aprenentatge associatiu fins a la complexa cognició humana (Cooke & Bliss 2006). D'altra banda, existeixen estudis que utilitzen els registres electrofisiològics *in vitro* per conèixer el substrat neuroquímic present en la formació de la memòria i troben que l'administració de diferents fàrmacs en les seccions de teixit pot modificar la PLT, bloquejant-la o afavorint-la. Els mecanismes de plasticitat sinàptica s'han observat en diverses estructures que participen en el processament de la memòria (Bliss & Collingridge 1993), com són l'HPC i el CPF o l'amígdala, regions en les quals ens centrarem en el present treball.
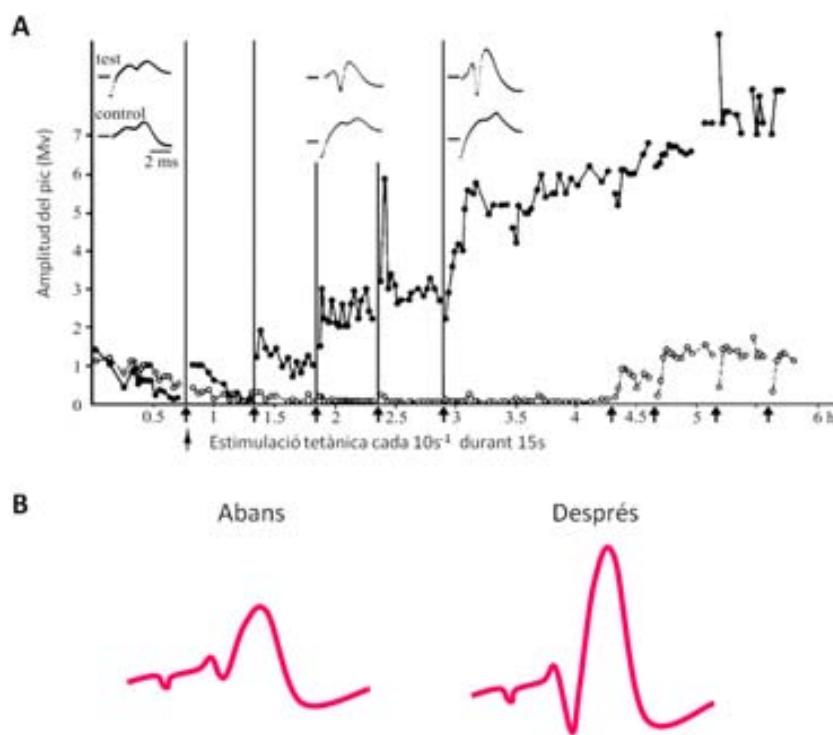
Figura 5. Gràfics de la potenciació a llarg termini. (A) Increment a llarg termini de l'eficiència de la transmissió en les sinapsis de les cèl·lules granulars del gir dentat induïda per l'estimulació tetànica de la via perforant. El gràfic mostra l'amplitud de resposta al llarg del temps (B) Representació del PEP abans i després de la inducció de la PLT a través de l'estimulació tetànica. (Adaptat de Bliss & Lomo 1973).

1.3.1   L'hipocamp

L'HPC és una estructura cerebral que es localitza en la regió medial del lòbul temporal d'ambdós hemisferis, ventral al cos callós, adjacent a l'amígdala i ocupa el terra del ventricle lateral. Forma part de l'anomenada formació hipocampal constituïda per quatre regions: l'HPC, el que inclou alhora tres subcamps, *cornus ammonis* CA1, CA2 i CA3, el gir dentat (GD) i el complex subicular, el qual inclou també tres subdivisions, el subicle (SUB), el presubicle i el parasubicle i l'escorça entorrinal (Figura 6).
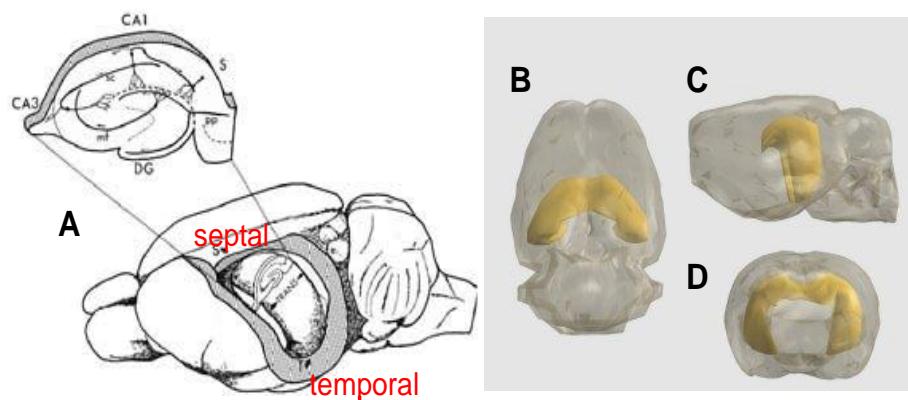
Figura 6. L'hipocamp de la rata. (A) Visió dorsolateral del cervell d'una rata on es mostra l'eix septotemporal i l'eix transversal (Adaptat d'Amaral & Witter 1989). (B) Visió dorsal, (C) Visió lateral i (D) Visió frontal de l'hipocamp de la rata (Adaptat de Lu et al. 2001).
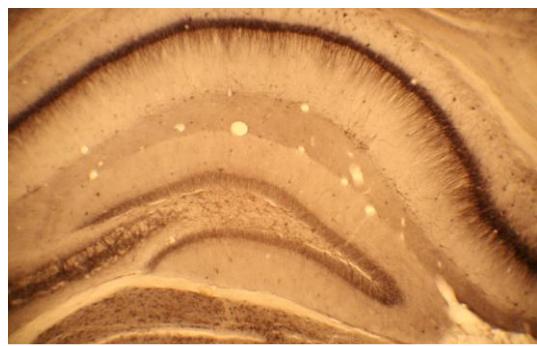
Figura 7. Estratificació cel·lular de l'hipocamp (Adaptat de Yague et al. 2010).

Des d'un punt de vista histològic, l'HPC es divideix en tres estrats cel·lulars: l'estrat polimorf (neurones GABAèrgiques intrínseques), l'estrat piramidal (neurones parvocel·lulars i magnocel·lulars) i l'estrat molecular (cèl·lules granulars) (Figura 7). En total està compost per un 90% de neurones glutamatèrgiques (cèl·lules granulars del GD i piramidals de l'HPC) i un 10% d'interneurones GABAèrgiques que modulen l'activitat de les cèl·lules glutamatèrgiques a través d'interaccions inhibitòries.

El principal circuit que connecta les cèl·lules hipocampals és l'anomenat circuit trisinàptic que està format, en primer lloc, per la via perforant que connecta l'escorça entorrinal amb el GD, en segon lloc, per la via molsosa que connecta el GD amb CA3 i, per últim, la via de les col·laterals de Schaffer que connecta CA3 amb les dendrites de CA1. Aquest circuit és el més rellevant de l'HPC i és

on s'ha estudiat exhaustivament la inducció i el manteniment de la PLT. No obstant això, també existeixen altres connexions, com per exemple, les establertes entre neurones de CA1 i l'escorça entorrinal i el SUB (Figura 8). Addicionalment també es troba la via fímbria-fòrnix que és el principal sistema d'aferències i eferències de caràcter subcortical de la formació hipocampal (Daitz & Powell 1954; Powell et al. 1957), i la que transporta fibres de l'HPC i el SUB cap els nuclis del septe, el nucli accumbens, els nuclis del tàlem i de l'hipotàlem (Canteras & Swanson 1992). A més a més, també transporta fibres aferents a la formació hipocampal, principalment *inputs* colinèrgics des del prosencèfal basal (PB) i el septe medial, noradrenèrgics del *locus coeruleus* i serotoninèrgics dels nuclis del rafe (Figura 9).
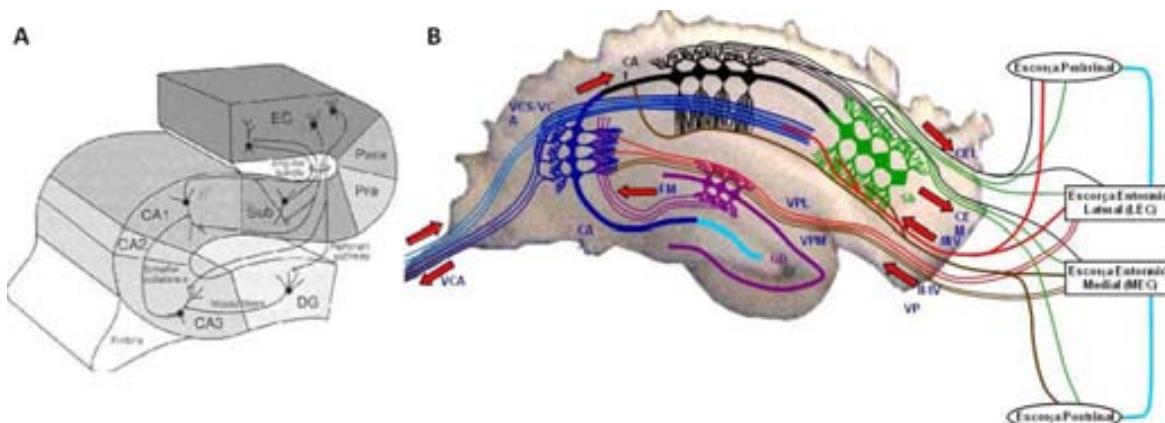
Figura 8. Circuits neuronals intra i extrahipocampals. (A) Secció de cervell humà (adaptat d'Andersen et al. 2007) (B) cervell de rata [VCA : via comissural ; CA1: subcamp de l'hipocamp CA1; CA2: subcamp de l'hipocamp CA2; CA3: subcamp de l'hipocamp CA3; GD: gir dentat; CEL: escorça entorrinal lateral ; CEM : escorça entorrinal medial; FM: fibres molsoses; III/V : capes III i V de l'escorça entorrnial; II/IV : capes II i IV de l'escorça entorrinal ; Sb : *subiculum* ; VCS/VCA : via comissural/associacional (col·laterals) de Schaffer ; VP : via perforant ; VPL : via perforant lateral ; VPM : via perforant medial] (Adaptat de www.unibristol.uk).

L'HPC no actua com una estructura unitària, ja que s'han descrit diferències en la connectivitat entre la part dorsal (HPCd) i la part ventral (HPCv) (Amaral & Witter 1989; Moser & Moser 1998) (Figura 10), que correlacionen amb les seves diferències funcionals (Fanselow & Dong 2010). En aquest sentit, les escorces d'associació visual, auditiva i somatosensorial, així com el còrtex perirrinal, projecten majoritàriament sobre l'HPCd a través de l'escorça entorrinal lateral, mentre que la informació olfactòria arriba de manera més extensa a l'HPCv (Amaral & Witter 1989; Moser & Moser 1998). D'altra banda, s'ha descrit que l'HPCv projecta directament al CPF, al bulb olfactori, a l'estria terminal, a l'amígdala i al nucli accumbens, així com a altres estructures associades a l'eix hipotalàmic-hipofisiari-suprarenal (Verwer et al. 1997; van Groen & Wyss 1990; Pitkänen et al. 2000; Amaral & Witter 1989). Aquesta connectivitat ha permès relacionar l'HPCv amb la regulació de les conductes emocionals, com l'estrès i l'ansietat (Hawley et al. 2012) i també amb els aprenentatges olfactoris (Martin et al. 2007). Per tant, les lesions o inactivacions específiques d'aquesta regió

provoquen un efecte ansiolític i una disminució de la resposta de por (Donley et al. 2005; Sutherland et al. 2008), un dèficit en tasques de memòria olfactòria (Ross & Eichenbaum 2006; Hunsaker et al. 2008) i les lesions neonatals s'han proposat com a model d'esquizofrènia (Lipska et al. 1993). D'altra banda, tot i que l'HPCd ha rebut una major implicació en relació amb l'aprenentatge espacial, en ambdues regions s'han localitzat cèl·lules de lloc (Jung et al. 1994; Poucet et al. 1994; Moser et al. 2008). Aquestes cèl·lules responen davant localitzacions específiques de l'entorn i permeten la codificació d'estímuls contextuals (Poucet et al. 2000). El seu descobriment va permetre desenvolupar la teoria del mapa cognitiu, segons la qual, la memòria espacial ve donada per la formació d'un mapa de l'entorn que tindria lloc a l'HPC (O'Keefe & Nadel 1978) i que guiaria la conducta d'orientació. Els rNMDA hipocampals modulen aquesta funció, ja que el seu bloqueig impedeix l'aprenentatge espacial (McDonald et al. 2005; Assini et al. 2009).

Les característiques descrites anteriorment fan de l'HPC una estructura molt rellevant en els processos de plasticitat sinàptica i, per tant, una regió molt implicada en els mecanismes d'aprenentatge i memòria (Annexos 1-2). Els mecanismes de PLT són fàcilment demostrables en aquesta regió, ja que el patró d'estimulació que indueix la PLT imita els ritmes theta naturals del propi HPC, enregistrats durant l'adquisició de diverses tasques conductuals (Rose & Dunwiddie 1986; Diamond 1990). A més, els diferents canvis bioquímics que es produeixen a l'HPC després de la PLT també es donen durant l'adquisició i la consolidació de la memòria (Lynch 2004). Com s'ha descrit, aquests mecanismes depenen principalment de l'activitat glutamatèrgica i, en específic, dels rNMDA, així que el bloqueig d'aquests impedeix l'aparició de la PLT i paral·lelament deteriora l'adquisició i la retenció de diverses tasques HPC dependents (Morris et al. 1986; Collingridge et al. 1983; Brun et al. 2001). La plasticitat que presenten les seves neurones fonamenta que sigui una estructura relacionada amb els processos d'emmagatzematge de memòria, especialment de memòria relacional o declarativa (Squire 1992; Squire et al. 2004; VanElzakker et al. 2008). L'HPC també s'ha relacionat amb altres processos cognitius com són la memòria de treball, la memòria sensoriomotora i el reconeixement d'estímuls nous. El substrat neurobiològic d'aquestes funcions es basa en les connexions de l'HPC amb nuclis del tronc de l'encèfal i amb el CPF (Vanderwolf & Cain 1994; Meunier & Barbeau 2013).
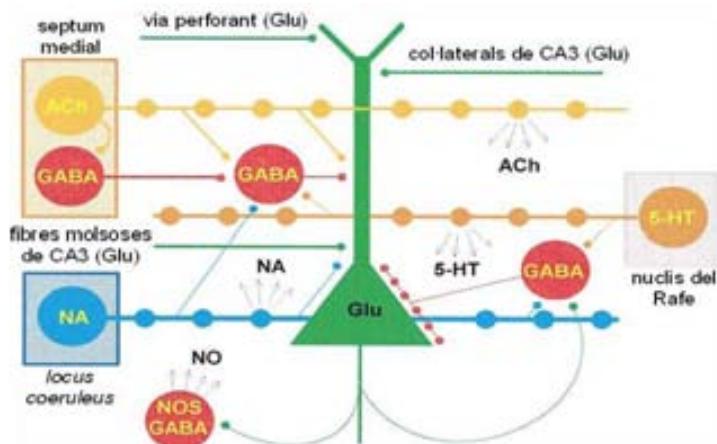
Figura 9. Diagrama de l'organització neuroquímica de la formació hipocampal. Les principals cèl·lules (piramidals i granulars) són glutamatèrgiques i les interneurones són GABAèrgiques. [5-HT : serotonina ; ACh : acetilcolina; Glu: glutamat; NA: noradrenalina; NO: òxid nítric; NOS: síntesi d'òxid nítric] (Adaptat de Vizi & Kiss 1998).
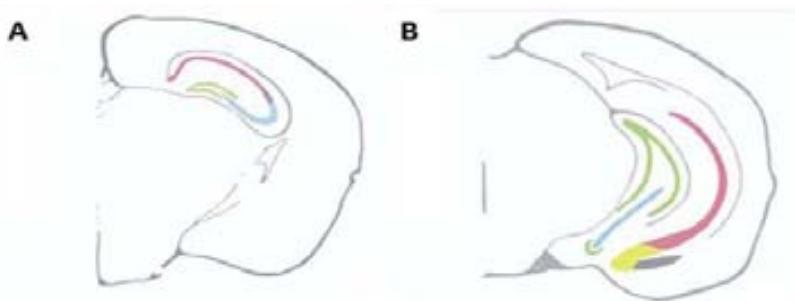
Figura 10. Visió esquemàtica de les diferències en l'estructura de la formació hipocampal en l'HPCd (A) i l'HPCv (B). En rosa CA1, en lila CA2, en blau CA3, en verd el GD i en groc el SUB (Adaptat de Leonardo et al. 2006).

1.3.2   El còrtex prefrontal

El CPF és la regió més rostral del lòbul frontal que, si bé presenta unes fronteres anatòmiques imprecises en les diferents espècies de mamífers, en totes elles posseeix una connectivitat recíproca amb el nucli mediodorsal del tàlem (Rose & Woolsey 1948). En humans el CPF constitueix el 30% de tota l'escorça cerebral i és l'àrea del cervell que té un desenvolupament ontogenètic i una mielinització més tardana. El CPF s'ha dividit en tres regions principals, la dorsolateral, l'orbitofrontal i la cingulada anterior, però en primats no humans, es divideix en dues regions, l'orbitomedial i la dorsolateral (Fuster 2000). En rates, el CPF es divideix generalment en tres regions topogràficament diferents: la lateral, la ventral i la medial (CPFm), la qual es subdivideix alhora en cinc regions (de dorsal a ventral): el còrtex precentral, el còrtex cingulat anterior, el CPL, el còrtex infralímbic (CIL) i el còrtex orbital medial (Figura 12). Segons diversos estudis anatòmics i funcionals, el CPL es considera,

en general, l'àrea homòloga a l'escorça prefrontal dorsolateral d'humans i primats no humans, encarregada principalment de les funcions executives (Granon et al. 1998; Vertes 2004).

Pel que fa a la citoarquitectura del CPF, en primats s'agrupa en sis capes horitzontals, essent algunes difícils de diferenciar en rates, en el cervell de les quals només se n'identifiquen quatre. A l'escorça, les neurones piramidals representen al voltant d'un 80% del total i són glutamatèrgiques, mentre que el 20% restant són interneurones GABAèrgiques que proporcionen un control local inhibitori. En general, les neurones s'organitzen en microcircuits múltiples i repetitius que inclouen les neurones piramidals i les seves connexions d'entrada i de sortida. Aquestes entrades provenen de les cèl·lules espinoses excitadores i de les interneurones GABAèrgiques inhibitòries, les quals estan interconnectades entre elles. Aquest microcircuit bàsic es va repetint en cada capa cel·lular, coordinant-se paral·lelament amb els microcircuits veïns (DeFelipe 2002). El CPF té una connectivitat complexa amb multitud d'estructures tant corticals com subcorticals, la qual correlaciona amb la seva implicació funcional en diversos processos cognitius, convertint-se en una escorça associativa que integra informació multimodal, incloent inputs sensorials, motors, cognitius, emocionals i autonòmics (Gabbott et al. 2005; Heidbreder & Groenewegen 2003; Vertes 2004) (Figura 12). Així doncs, el CPL és una de les principals regions corticals de projecció colinèrgica del PB (Gaykema et al. 1990; Saper 1984; Van Eden et al. 1992) i té connexions recíproques amb diferents estructures olfactòries (Berendse et al. 1992; Datiche & Cattarelli 1996, Neafsey et al. 1986), amb la formació hipocampal, amb l'amígdala (Carr & Sesack 1996; Gabbott et al. 2002; McDonald 1987; McDonald 1991) i amb nuclis del tronc de l'encèfal (Vertes 2004).
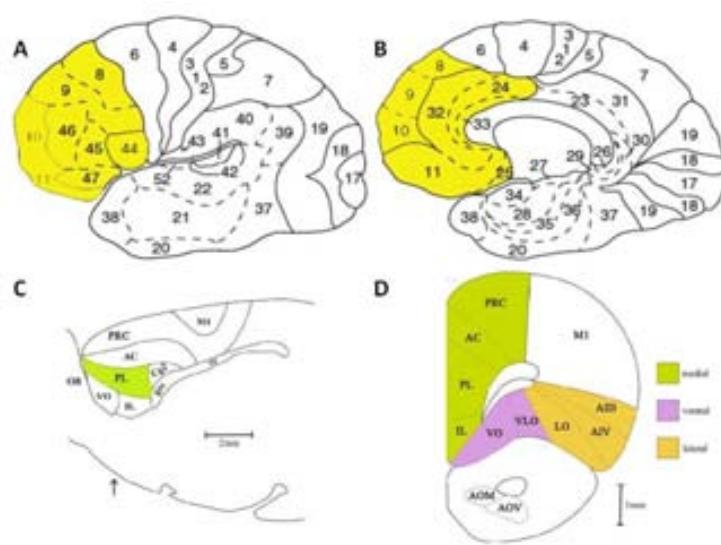
Figura 11. El còrtex prefrontal. (A, B) Mapa citoarquitectònic de l'escorça del cervell humà segons Brodmann. Visió lateral (A) i visió medial (B) (C, D) Estructura de l'escorça prefrontal de la rata. Secció sagital (C) i secció coronal (D) [AC: còrtex cingulat anterior; AID: còrtex insular agranular part dorsal; AIV: còrtex insular agranular part ventral; AOM: nucli olfactori anterior part medial; AOV: nucli olfactori anterior part ventral; Cg2: còrtex cingulat àrea 2; cc: cos callós; gcc: genoll del cos callós; IL: còrtex infralímbic; LO: còrtex orbital lateral; M1: còrtex motor primari; OB: bulb olfactori; PRC: còrtex precentral; PL: còrtex prelímbic; VLO: còrtex orbital ventrolateral; VO: còrtex orbital ventral] (Adaptat de Dalley et al. 2004a).
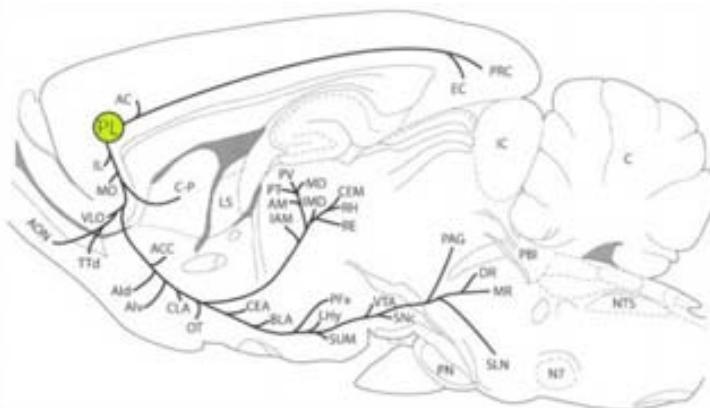
Figura 12. Secció sagital que mostra les principals projeccions del còrtex prelímbic. [AC: còrtex cingulat anterior; ACC: nucli accumbens; AId: còrtex insular agranular part dorsal; AIv: còrtex insular agranular part ventral; AM: nucli anteromedial del tàlem; AON: nucli olfactori anterior; ABL: nucli basolateral de l'amígdala; C: cerebel; C-P: nuclis caudat i putamen, estriat; CEA: nucli central de l'amígdala; CEM: nucli central medial del tàlem; CLA: claustrum; EC: còrtex entorrinal; DR: nucli dorsal del rafe; IAM: nucli interanteromedial del tàlem; IC: col·licle inferior, IL: còrtex infralímbic; IMD: nucli intermediodorsal del tàlem; LHy: àrea hipotalàmica lateral; LS: nucli septal lateral; MD: nucli mediodorsal del tàlem; MO: còrtex orbital medial; MR: nucli mitjà del rafe; NTS: nucli del tracte solitari; N7: nucli facial; OT: tubercle olfactori; PAG: substància grisa periaqüeductal; PBl: nucli parabraquial lateral; PFx: regió perifornical de l'hipotàlem; PL: còrtex prelímbic; PN: nucli pontí; PRC: còrtex perirínic; PT: nucli parataenial del tàlem; PV: nucli paraventricular del tàlem; RE: nucli reuniens del tàlem; RH: nucli romboide del tàlem; SLN: nucli supralemniscal; SNc: Substància negra, *pars compacta*; SUM: nucli supramamil·lar; TTd: taenia tecta dorsal; VLO: còrtex orbital ventrolateral; VTA: àrea tegmental ventral] (Adaptat de Vertes 2004).

El CPF està relacionat funcionalment amb l'aparició de capacitats associades a la cognició, com són la memòria i les funcions executives (Annexos 3-4). Les implicacions funcionals d'aquesta regió s'han estudiat bàsicament mitjançant estudis de lesió que solen deteriorar la consolidació de la memòria a llarg termini (Laroche et al. 2000), així com produir dèficits en la memòria de treball, la flexibilitat cognitiva, la inhibició conductual, la detecció de contingències i els processos atencionals (Brito & Brito 1990; Delatour & Gisquet-Verrier 1996; Jinks & McGregor 1997; Killcross & Coutureau 2003; Passetti et al. 2002; 2003; Taylor et al. 2003). A més a més, s'ha descrit que la disfunció d'aquesta regió i dels circuits associats provoca molts dels dèficits descrits en trastorns neuropsiquiàtrics com el dèficit d'atenció i hiperactivitat, l'esquizofrènia, l'ansietat i la malaltia d'Alzheimer (Dalley et al. 2004a; Schroeter et al. 2012). Les funcions del CPF depenen del correcte funcionament de la transmissió glutamatèrgica, ja que l'administració d'antagonistes dels rNMDA en aquesta regió bloqueja de forma significativa l'execució de tasques executives i la consolidació de la memòria (Roberts et al. 2010; Wang et al. 2013; van Windergen et al. 2012; Moghaddam & Jackson 2003; Akirav & Maroun 2006; Takehara-Nishiuchi et al. 2005).

A nivell cel·lular, el CPL, va ser una de les primeres regions on es va descriure la PLT fora de l'HPC, i es va observar, en el fascicle que connecta l'HPC amb el CPL (Doyere et al. 1993). Des

d'aleshores, també altres estudis han descrit l'existència de PLT a altres vies corticals (Ling et al. 2002; Vickery et al. 1997; Haul et al. 1999; Jay et al. 1996). Per tant, els fenòmens de plasticitat sinàptica en aquesta regió podrien explicar la seva implicació en l'aprenentatge i la memòria (Jay et al. 1996) i les seves connexions amb l'HPC la seva participació en la formació de la memòria relacional (DeVito et al. 2010).

1.3.3 L'amígdala

Una altra de les regions cerebrals relacionades amb processos de memòria estudiada en el present treball és l'amígdala. L'amígdala és un conjunt heterogeni de nuclis situat al lòbul temporal que limita al llarg de la seva extensió rostro-caudal amb l'escorça, l'HPC, l'estriat i els ventricles laterals (Gray & Bingaman 1996). Els nuclis de l'amígdala es poden dividir en tres grups, els nuclis profunds, amb importants connexions amb el còrtex, els nuclis superficials, directament connectats amb el sistema olfactori, i altres nuclis connectats amb regions de control autonòmic de l'hipotàlem lateral i del tronc de l'encèfal (Price et al. 1987) (Figura 13). Pel que fa a la seva citoarquitectura, s'ha descrit que presenta certes similituds amb el CPF, ja que podem diferenciar dues poblacions neuronals: les cèl·lules piramidals espinoses o de classe I (McDonald 1982; 1984) que en són la població majoritària, i les cèl·lules no espinoses o de classe II (Millhouse & DeOlmos 1983). En aquest cas, les cèl·lules piramidals, considerades el principal output de l'amígdala, són eminentment glutamatèrgiques (Smith & Paré 1994; Farb & LeDoux 1997; 1999) mentre que les interneurones, presents a la majoria de nuclis, són fonamentalment GABAèrgiques (Roberts 1992; Price et al. 1987).

Així doncs, la informació sensorial arriba al complex amigdalí a través dels nuclis laterals, on es processa inicialment, per després enviar-la cap a altres nuclis seguint una progressió predominantment lateromedial cap als nuclis basals, on la informació és reprocessada i avaluada amb major complexitat, per enviar-la finalment cap als nuclis centromedials que actuen com a sortida de la informació (Sah et al. 2003) (Figura 14). L'amígdala presenta connexions recíproques amb diferents estructures telencefàliques com la formació hipocampal, l'escorça cerebral o els ganglis basals, amb estructures diencefàliques com el tàlem i l'hipotàlem, així com amb estructures del tronc de l'encèfal i, fins i tot, amb regions de la medul·la espinal. Les vies que connecten l'amígdala amb les estructures subcorticals viatgen a través de dos feixos de fibres que contenen tant eferències com aferències (Price et al. 1987), la via amígdalofugal ventral i l'estria terminal, mentre que les projeccions corticals es realitzen a través de la càpsula externa (LeDoux 1992a).
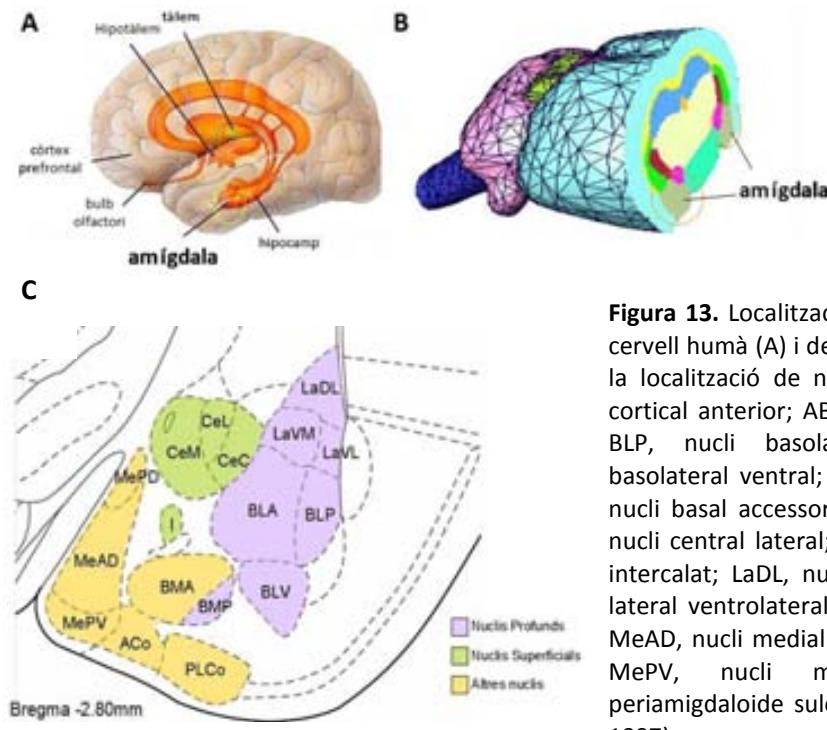
Figura 13. Localització esquemàtica de l'amígdala en el cervell humà (A) i de rata (B). Diagrama i classificació de la localització de nuclis de l'amígdala (C) [ACo: nucli cortical anterior; ABL, nucli basolateral magnocel·lular; BLP, nucli basolateral parvicel·lular; BLV, nucli basolateral ventral; BMA, nucli cortical anterior; BMP, nucli basal accessori; CeC, nucli central capsular; CeL, nucli central lateral; CeM, nucli central medial; I, nucli intercalat; LaDL, nucli lateral dorsolateral; LaVL, nucli lateral ventrolateral; LaVM, nucli lateral ventromedial; MeAD, nucli medial dorsal; MePD, nucli medial caudal; MePV, nucli medial ventral; PLCo, còrtex periamigdaloide sulcal] (Adaptat de Paxinos & Watson 1997).

El conjunt de nuclis amigdalins participa en diverses funcions cognitives (Annexos 5-6), amb un paper principal en els processos emocionals (Amaral et al. 1992; Everitt & Robbins 1992; LeDoux 1992a; 1992b; 1993; 1995; 2000; 2003; Murray 2007; Fanselow & Gale 2003; Davis 1994; McGaugh 2004; Ritchey et al. 2008). Si bé l'amígdala lateral i l'ABL són nuclis especialment rellevants en el condicionament de la resposta de por (Davis 1992a; 1992b; 1994; Tazumi & Okaichi 2002; Everitt et al. 1991), també s'han vist implicats en l'aprenentatge i el record d'estímuls apetitius (Hamann et al. 1999). Els rNMDA de l'ABL són crítics per l'aprenentatge d'extinció de la por, ja que s'observen dèficits quan es bloquegen i millora quan es potencien (Falls et al. 1992; Laurent & Westbrook 2010; Zimmerman & Maren 2010; Walker et al. 2002). No obstant, el rol d'aquests receptors en l'extinció de tasques apetitives ha estat menys investigat i ha generat resultats divergents. El seu bloqueig abans de l'extinció accelera l'aprenentatge d'extinció posterior, mentre que la inactivació durant l'extinció provoca un dèficit d'aquest aprenentatge (Sun & Laviolette 2012). Aquest fet podria ser degut a què els rNMDA de l'ABL també participen en la reconsolidació de la memòria. Així doncs, sembla que en funció del moment en què es dugui a terme la manipulació es poden obtenir efectes diferents. L'ABL es relaciona també amb l'aprenentatge olfactori i gustatiu, independentment del seu caràcter apetitiu o aversiu, donat que és una de les regions que rep importants projeccions des del còrtex piriforme i des dels nuclis parabraquials i el còrtex insular (Paré 2003). D'altra banda, estudis de registre electrofisiològic han observat respostes de l'ABL tant a estímuls gustatius (Yasoshima et al. 1995; Nishijo et al. 1998) com a estímuls olfactoris (Rosenkranz & Grace 2002; Sevelinges et al.

2004). Una vegada més, el bloqueig dels rNMDA provoca una alteració en l'aprenentatge de tasques de naturalesa olfactòria (Hatfield & Gallagher 1995; Walker et al. 2005; Ferry & Di Scala 2000).
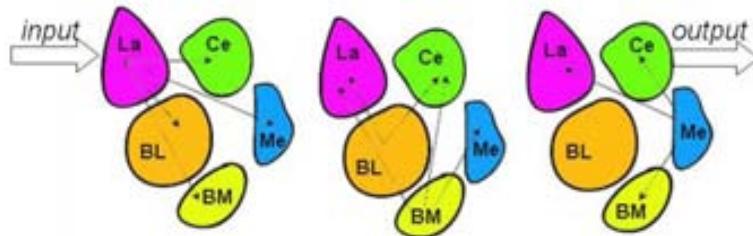
Figura 14. Esquema de les principals connexions entre els nuclis amigdalins [BL, nucli basolateral; BM, nucli basal accessori; Ce, nucli central; La, nucli lateral; Me, nucli medial] (Adaptat de Sah et al. 2003)

De manera similar a l'HPC i al CPL, a l'amígdala també s'han contemplat fenòmens de plasticitat neural, com la PLT (Maren & Fanselow 1996; Pape & Stork 2003; Nacher et al. 2002; Guterman & Richter-Levin 2006). S'ha descrit que l'activació dels rNMDA de l'ABL permet la síntesi de noves proteïnes i fenòmens de transcripció regulats per CREB, els quals són fonamentals per a la consolidació, la reconsolidació i l'extinció de la memòria (Nader et al. 2000). Les eferències de l'amígdala a l'HPC i a l'escorça permeten que l'amígdala tingui un paper modulador en la plasticitat d'aquestes, trobant-se, per exemple, que l'activació de l'ABL afavoreix la PLT de l'HPC i transforma una PLT primerenca a una PLT tardana (Akirav & Richter-Levin 1999). Per tant, és congruent que el bloqueig dels rNMDA de l'amígdala provoqui una alteració en el record de diverses tasques (Walker et al. 2005; LaLumiere et al. 2004) i eviti el procés de reconsolidació de la memòria (Milton et al. 2008), mentre que una potenciació d'aquests receptors faciliti la reconsolidació de la memòria (Lee et al. 2009).

1.4   Modulació dels receptors NMDA i processos d'aprenentatge i memòria

Com s'han mencionat anteriorment, els rNMDA són una diana interessant pel disseny de tractaments destinats a la millora cognitiva, ja que estan íntimament implicats en els processos cognitius i la seva activació és un detonant per a la inducció de la PLT (Collingridge et al. 1983; Rebola et al. 2010). A més a més, s'ha observat una disminució en el nombre d'aquests receptors en el cervell de rates causada pel procés de l'envelliment, la qual podria explicar alguns dels dèficits cognitius associats a l'edat (Barnes 1979; Wenk & Barnes 2000), i un augment en el cervell de rates amb major capacitat d'aprenentatge (Stecher et al. 1997). Malgrat això, una excessiva activació d'aquests receptors podria originar condicions patològiques, tals com la mort neuronal (Simon et al.

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1984) o alteracions com l'epilèpsia (Croucher et al. 1982) o la hiperalgèsia (Davies & Lodge 1987). Aquest fet suggereix que, el camí més adient per aconseguir una facilitació cognitiva consisteix a estimular la seva funció fisiològica sense promoure conseqüències patològiques.

Les propietats dels rNMDA permeten diverses formes de modulació indirecta, la majoria de les quals s'utilitzen fisiològicament amb la finalitat de dissenyar fàrmacs que permetin la potenciació cognitiva (Figura 15). S'ha descrit que una via adequada per potenciar la funció dels rNMDA és actuant sobre els altres receptors glutamatèrgics, ja sigui enfortint la despolarització a través de l'activació dels rAMPA (Knafo et al. 2012), o dels receptors metabotròpics (Vinson & Conn 2012), ja que ambdues modulacions potencien la inducció de la PLT (Collingridge 1985; Kroker et al. 2011) i milloren els processos d'aprenentatge i memòria, tant en humans (Morrow et al. 2006) com en animals (Darrah et al. 2008).
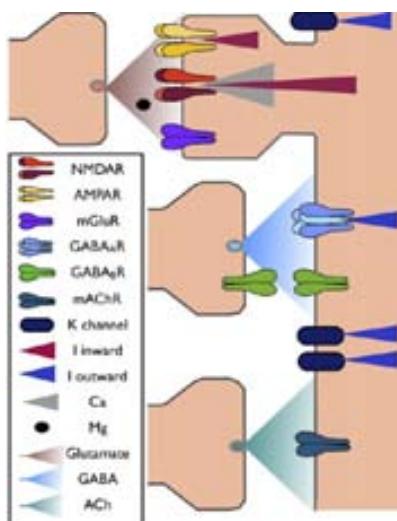
Figura 15. Modulació indirecta dels receptors NMDA. Representació esquemàtica de com la funció del receptor NMDA pot ser regulada a través de diferents neurotransmissors i altres reguladors neuronals (Adaptat de Collingridge et al. 2013).

D'altra banda, s'ha demostrat que la modulació d'altres sistemes de neurotransmissió també pot afectar l'activació dels rNMDA. Per exemple, s'ha descrit que l'activació dels receptors GABA impedeix que els rNMDA participin en la resposta sinàptica, ja que augmenta la hiperpolarització de la neurona i intensifica el bloqueig del canal causat pel Mg^{2+} (Herron et al. 1985). Per tant, l'administració d'antagonistes GABAèrgics augmenta la inducció de PLT i millora dèficits cognitius (Olpe et al. 1993). També s'ha postulat que l'activació dels receptors colinèrgics facilita els processos de plasticitat cerebral dependents dels rNMDA i potencia els processos cognitius (Sarter & Parikh, 2005). Així doncs, una modulació del sistema colinèrgic pot augmentar la probabilitat de què un estímul excitador arribi al llindar d'activació dels rNMDA (Jo et al. 2010; Collindridge & Singer 1990).

Tanmateix, el rNMDA es pot modular mitjançant l'administració de substàncies que s'hi uneixin directament. L'administració d'antagonistes suscita una regulació cortical defectuosa del Glu, alterant els processos d'aprenentatge i memòria en algunes tasques (Maurice et al. 1994; Moghaddam et al. 1997; Parada-Turska & Turski 1990). Així, l'administració sistèmica de ketamina, dizocilpina (MK-801), fenciclidina o àcid D,L-2-amino-5-fosfonovaleric (APV) provoca dèficits generalitzats en diferents processos cognitius i en diversos tipus de tasques. El bloqueig d'aquests receptors deteriora les funcions executives com la memòria de treball, la inhibició conductual i els processos atencionals (Kawabe et al. 2007; Jentsch et al. 1997, 1998; Murphy et al. 2005; Pozzi et al. 2011; Verma & Moghaddam 1996). Així com també provoca alteracions tant en l'aprenentatge com en el record de paradigmes de memòria implícita (Smith et al. 1997; Zimmerman et al. 2010; Ranaldi et al. 2011; Carmack et al. 2013), d'aprenentatge espacial o relacional (Murphy et al. 2005; Patel et al. 1998; Roberts & Shapiro 2002; Cestari & Castellano 1997; Morris 1989; Liang et al. 1994; Packard & Teather 1997; McDonald et al. 2005), d'aprenentatges olfactoris i de memòria social (Stäubli et al. 1989; Storozheva et al. 2011; Gao et al. 2009). No obstant això, els antagonistes dels rNMDA podrien ser també considerats com a potenciadors cognitius sota certes circumstàncies. L'exemple més rellevant és la memantina, un fàrmac utilitzat pel tractament de la malaltia d'Alzheimer, el qual té un efecte moderat en el retard del declivi cognitiu (Danysz & Parsons 2003). Una de les maneres a través de les quals aquest fàrmac pot ser capaç de millorar la cognició és inhibint selectivament l'activació patològica i preservant l'activació fisiològica dels rNMDA (Collingridge et al. 2013).

1.5 La D-cicloserina: un modulador positiu dels receptors NMDA

Com s'ha comentat anteriorment, per obrir el canal del rNMDA, a part del Glu, es necessita que el coagonista glicina s'uneixi al seu lloc d'unió (Johnson & Ascher 1987; McBain et al. 1989). Si bé l'NMDA potencia les funcions cognitives quan s'administra de manera sistèmica (Hlinak & Krejcí 2002; Koek et al. 1990), la majoria d'estudis utilitza agonistes del lloc d'unió de la glicina per tractar diverses patologies i potenciar la cognició amb l'objectiu d'evitar possibles efectes de toxicitat dels agonistes directes (Jansen & Dannhardt 2003). Un dels agonistes que més s'ha utilitzat en models animals i en assajos preclínics amb humans per revertir dèficits cognitius o bé millorar els processos d'aprenentatge i memòria és la DCS (Monahan et al. 1989; Henderson et al. 1990).

La DCS, D-4-amino-3-isoxazolidona, és un agonista parcial dels rNMDA que actua al lloc d'unió de la glicina i potencia l'activitat glutamatèrgica de manera substancial (Hood et al. 1989; Normann & Berger 2008) (Figura 16). Essent un antibiòtic, inicialment es va usar per al tractament de la tuberculosi, però en els darrers anys s'ha utilitzat com a potenciador cognitiu degut a què pot millorar l'aprenentatge i la memòria (Monahan et al. 1989). En diversos experiments amb humans i

animals s'ha pogut constatar que aquest fàrmac no només és capaç de potenciar els processos cognitius, sinó que també reverteix el deteriorament de l'adquisició i el record de diferents aprenentatges produït per patologies o manipulacions experimentals com explicarem més endavant (veure apartat 1.5.2).
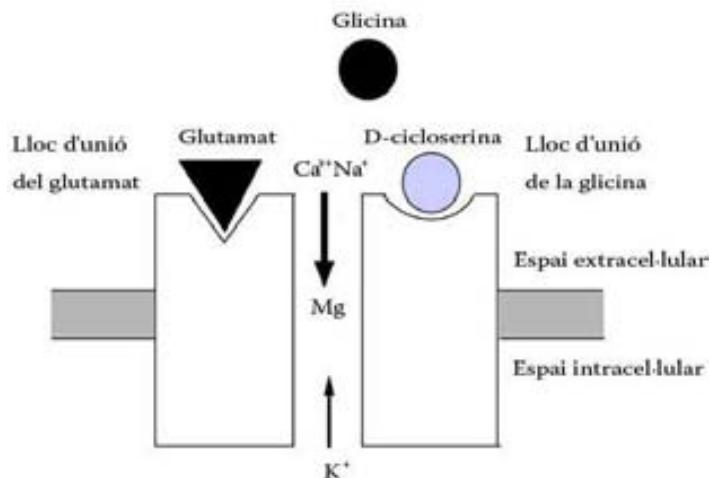
Figura 16. Representació esquemàtica del receptor NMDA coactivat per la DCS. La DCS actua al lloc d'unió de la glicina i permet l'obertura dels canals de cations (Adaptat de Norberg et al. 2008).

1.5.1   D-cicloserina com a potenciador cognitiu

a) Extinció i reconsolidació de la memòria

Estudis preclínics en humans mostren que la DCS, combinada amb teràpies conductuals d'exposició, podria constituir un tractament eficaç dels trastorns d'ansietat (Hofmann et al. 2013), ja que millora l'aprenentatge d'extinció de la resposta de por (Vervilet 2008). Aquest aprenentatge es refereix a la reducció del nivell de por mostrat (resposta condicionada, RC) davant d'un senyal (estímul condicionat, EC) prèviament aparellat amb un esdeveniment aversiu (estímul incondicionat, EI) quan aquest és presentat repetidament en absència de l'estímul aversiu. En animals, les mesures de por que més s'utilitzen són la petrificació i l'augment de la resposta d'ensurt, que disminueixen progressivament durant el procés d'extinció davant l'EC. Diversos experiments suggereixen que aquest aprenentatge no consisteix en un oblit de l'associació entre l'EC i l'EI, ja que la RC és resistent al pas del temps, sinó que es tracta d'un procés actiu d'aprenentatge inhibitori que requereix entrenament addicional perquè es pugui produir. Pel que fa als mecanismes neuroquímics subjacents al procés d'extinció, s'ha demostrat que depèn especialment dels rNMDA (Myers & Davis 2007) i així ho corroboren diversos estudis que han utilitzat substàncies agonistes i antagonistes d'aquests receptors. Tenint en compte els estudis amb la DCS, cal destacar que els efectes obtinguts són

diferents segons la forma d'administració, sistèmica o injectada directament al cervell, el moment en què s'administra, abans o després de l'entrenament d'extinció, i el tipus de tasca avaluada, apetitiva o aversiva (Taula 1).

La majoria de treballs que han investigat els efectes de l'administració sistèmica d'aquest fàrmac en tasques aversives, principalment en el condicionament de la por i de l'aversió pel lloc, mostren que l'administració de DCS abans i després de l'entrenament pot facilitar l'aprenentatge d'extinció i la consolidació de la nova traça de memòria d'aquests paradigmes (Preentrenament: Bouton et al. 2008; Langton & Richardson 2008; Lin et al. 2010; Myers & Carlezon 2010; Woods & Bouton 2006; Yamamoto et al. 2008; Yang et al. 2007; Langton & Richardson 2008; Lehner et al. 2010; Postentrenament: Ledgerwood et al. 2005; Vervliet 2008; Weber et al. 2007; Gupta et al. 2013). Un menor nombre d'estudis, però, ha investigat els efectes de la DCS en tasques apetitives i en la majoria d'ells s'han utilitzat substàncies addictives. En general, aquests experiments han observat que quan la DCS s'administra després de l'extinció es produeix una millora de la consolidació de l'extinció (Kelley et al. 2007; Paolone et al. 2009). En canvi, quan s'administra abans de l'entrenament els resultats generen certa controvèrsia; en alguns experiments la DCS potencia l'aprenentatge d'extinció (Nic Dhonnchadha et al. 2010; Vengeliene et al. 2008), mentre que en d'altres no causa cap efecte (Groblewski et al. 2009; Flavell et al. 2011; Vurbic et al. 2011) o bé, fins i tot, el dificulta (Port & Seybold 1998). Aquestes diferències es podrien explicar per la durada i quantitat d'assajos realitzats durant l'entrenament d'extinció, ja que si el nombre d'assajos és breu, el procés que es podria activar és el de reconsolidació.

En el procés d'extinció s'han implicat diverses estructures cerebrals, com per exemple el còrtex sensorial, la substància grisa periaqüeductal, el septe lateral i els nuclis de l'estria terminal. No obstant això, les regions més investigades han estat l'amígdala, el CPF i l'HPC (Myers & Davis 2002). L'amígdala és una regió crítica per a l'adquisició de respostes condicionades, especialment de por, i sembla ser molt rellevant per a l'extinció d'aquest aprenentatge. Concretament, l'ABL ha estat l'estructura cerebral que ha rebut una major consideració en la literatura i en la qual ens centrarem en aquesta tesi doctoral. D'altra banda, el CPFm, particularment el CIL i el PLC, és una àrea estretament lligada a l'ABL (McDonald 1998) pel que també participa en l'extinció, mentre que l'HPC està implicat, especialment, quan es tracta d'un aprenentatge de por associat al context (Myers & Davis 2007; Myers & Davis 2002). Així doncs, diversos estudis han utilitzat l'administració intracerebral de DCS per avaluar els seus efectes en l'extinció de diferents aprenentatges. En els paradigmes aversius, la DCS injectada a l'ABL, tant pre com postentrenament, (Ledgerwood et al. 2003; Mao et al. 2006; Walker et al. 2002), i preentrenament a l'HPC (Ren et al. 2013), afavoreix

l'extinció del condicionament de la resposta de por. Un dels mecanismes neuroquímics que podria explicar els efectes de la DCS seria l'augment de l'expressió de la subunitat GABA-A alpha-2 dels receptors gabaèrgics i de la concentració local de GABA a l'amígdala (Lehner et al. 2010; Wislowska-Stanek 2011), que facilitaria l'aprenentatge inhibitori. Així com també, un increment de l'expressió de rNMDA a l'HPC que correlacionaria amb la plasticitat cerebral subjacent a l'adquisició d'un nou aprenentatge (Ren et al. 2013). D'altra banda, un menor nombre d'estudis ha investigat els efectes de la DCS intracerebral en paradigmes apetitius, i en l'ABL només s'ha investigat l'administració postentrenament. En general, sembla que la DCS podria millorar l'extinció d'aquestes tasques (Botreau et al. 2006), però també hi ha estudis amb resultats contradictoris (Torregrossa et al. 2010). De la mateixa manera, si bé la literatura en humans demostra que aquest fàrmac podria ser d'utilitat en tractaments d'exposició (Hofmann et al. 2013), s'ha de tenir present que en funció del tipus de teràpia i moment d'administració es poden obtenir resultats contraproduents (Taula 1). Per exemple, estudis recents en pacients amb dependència a l'alcohol o la cocaïna han descrit que l'administració de DCS abans de la teràpia d'exposició no facilita el procés d'extinció (Watson et al. 2011), sinó que augmenta el desig associat a la droga (Hofmann et al. 2012; Prisciandaro et al. 2013; Price et al. 2012; Price et al. 2009).

El procés de reconsolidació és un procés diferent del d'extinció ja que, en aquest cas, la reactivació breu de la memòria d'un aprenentatge ja consolidat fa que aquesta es torni susceptible a possibles modificacions. Aquesta reactivació es duu a terme mitjançant una reexposició a l'EC o al mateix ambient on es va produir l'aprenentatge però en absència de l'EI. Així doncs, els assajos de reactivació són molt similars als assajos de l'entrenament d'extinció, però són d'una durada i nombre inferiors (Sara et al. 1999). Aquest procés depèn especialment dels rNMDA (Lee & Everitt 2008), així doncs, tal com mostren els resultats de diversos experiments, la DCS injectada a l'ABL o de forma sistèmica abans de la reactivació facilita la reconsolidació del condicionament de la resposta de por augmentant la resposta de petrificació (Lee et al. 2006; Yamada et al. 2009; Bustos et al. 2010) i també la reconsolidació de l'addicció a la cocaïna (Lee et al. 2009).

Taula 1. Efectes de la D-cicloserina en els processos d'extinció i reconsolidació en animals i en teràpies d'exposició en humans

MOMENT	ADMINISTRACIÓ	RESULTATS	AUTORS
MODELS ANIMALS			
Condicionament de la resposta de por associada a un estímul			
30' pre	Sistèmic	↑ Extinció	Walker et al. 2002
20' pre	ABL		
15' pre	Sistèmic	↑ Extinció = Renovació	Woods & Bouton 2006
30' pre	ABL	↑ Extinció	Mao et al. 2006
20' pre	Sistèmic	↑ Extinció	Lee, Milton & Everitt, 2006
	ABL	↑ Reconsolidació	
15' pre	Sistèmic (+metirapona)	↑ Extinció	Yang et al. 2007
Immed. pre	Sistèmic	↑ Extinció = Re Extinció	Langton & Richardson 2008
30' pre	Sistèmic	↑ Extinció	Lin et al. 2009
15' pre / 15' post	Sistèmic	↑ Extinció	Ledgerwood et al. 2003
1' post	ABL		
Immed. post	Sistèmic	↑ Extinció = Reaprenentatge ↑ Generalització	Ledgerwood et al. 2005
Immed. post	Sistèmic	↑ Extinció	Parnas et al. 2005
Immed. pre i post	Sistèmic	= Extinció	
2 o 28 dies pre	Sistèmic	↑ Extinció	
	Sistèmic	= Extinció	
Immed. post	Sistèmic	↑ Extinció = Re Extinció	Weber et al. 2006
30' pre	Sistèmic	↑ Reconsolidació	Bustos et al. 2010
10' post		↑ Extinció	
24h post		↑ Extinció	Langton & Richardson 2010
	Sistèmic		
Immed. pre		= Extinció	
Immed. post	Sistèmic	↑ Retenció extinció	McCallum et al. 2010
40' pre	Sistèmic	↑ Extinció	Toth et al. 2012
Immed post			
20' pre	Sistèmic	↑ Extinció en animal amb estrés ↓ Extinció en animals sans	Saito et al. 2012
10' pre	HPC	↑ Extinció ↑ rNMDA2B a l'HPC	Ren et al. 2013
Condicionament de la resposta de por associada al context			
15' pre			Bouton et al. 2008
	Sistèmic	↑ Extinció	Myers & Carlezon, 2009
Immed. pre			
15' pre	Sistèmic / ABL	↑ Reconsolidació	Yamada et al. 2009
30' pre	ABL	↑ Extinció	
Immed. post	ABL	Reverteix dèficit en extinció induït per estrés	Akirav et al. 2009
6 dies durant	Sistèmic	Reverteix dèficit en extinció induïts per estrés Reverteix augment de RNMDA a l'HPC	Yamamoto et al. 2008
Immed. post	Sistèmic	↑ Extinció	Bertotto et al. 2010
30' pre	Sistèmic	↑ Extinció ↑ GABA a ABL	Lehner et al. 2010
Immed. post	Sistèmic	↑ Extinció ↑ pERK al CPFm	Gupta et al. 2013

Preferència pel lloc associat amb alcohol / cocaïna / morfina o autoadministració			
60' pre	Sistèmic	↑ Extinció	Vengeliene et al. 2008
Immed. pre durant	Sistèmic	= Extinció ↓ Recondicionament	Groblewski et al. 2009
30' pre Immed. post	Sistèmic	↑ Extinció ↓ Recondicionament	Nic Dhonnchadha et al. 2009
Immed. post	Sistèmic	↑ Extinció	Kelley et al. 2007
Immed. post	Sistèmic	↑ Extinció	Paolone et al. 2008
Immed. post	Sistèmic ABL	↑ Extinció	Botreau et al. 2006
Immed. post	Sistèmic	↑ Extinció	Thanos et al. 2009
20' pre	ABL	↑ Reconsolidació	Lee et al. 2009
Immed. pre	Sistèmic	↑ Extinció	Myers & Carlezon 2010
Immed. post	Sistèmic Nucli Acc ABL, HPC, CPL	↑ Extinció ↑ Extinció = Extinció	Torregrossa et al. 2010
Immed. pre	CIL	= Extinció	Chang & Maren 2011
20' pre	Sistèmic	= Extinció	Lu et al. 2011
Immed. post	Sistèmic	↑ Extinció	Thanos et al. 2011
Conducta reforçada amb menjar / sucrosa			
Immed. post	Sistèmic	↑ Extinció	Shaw et al. 2009
60' pre	Sistèmic	= Extinció	Flavell et al. 2011
30' pre	Sistèmic	↓ Extinció	Port & Seybold 1998
15' pre	Sistèmic	= Extinció	Vurbic et al. 2011
Immed. post	Sistèmic	↑ Extinció en C57BL/6 mice	Leslie et al. 2012
Immed. post	CIL	↑ Extinció	Peters & De Vries 2013
Conducta d'aversió al menjar			
Immed. post	Amígdala	= Extinció	Akirav et al. 2009
Immed. post	Sistèmic	↑ Extinció ↓ Recuperació espontània	Mickley et al. 2012

MOMENT	RESULTATS	AUTORS
TERÀPIES EN HUMANS		
Fòbies		
2h pre	↑ teràpia d'exposició (acrofòbia)	Ressler et al. 2004
pre i post	= tractament aracnofòbia	Guastella et al. 2007a
1h pre	↑ tractament fòbia social	Hofmann et al. 2006
2h pre		Guastella et al. 2007b
1h pre	= extinció a la por condicionada	Klumpers et al. 2012
	↑ teràpia en el trastorn de pànic	Otto et al. 2009
1h pre	↑ activació còrtex prefrontal ventromedial, orbitofrontal i cingulat anterior	Nave et al. 2012
post	↑ tractament exposició	Smits et al. 2013
post	= tractament aracnofòbia	Tart et al. 2013
TEPT / TOC		
30' pre	= extinció ↑ simptomatologia	Litz et al. 2012
	↑ tractament	Choi et al. 2010
TOC		
1h pre	↑ tractament	Storch et al. 2010

Dependència a drogues		
2h pre	= extinció en dependència alcohol	Watson et al. 2011
15' pre	= extinció en dependència cocaïna ↑ *craving*	Kimber et al. 2012 Prisciandaro et al. 2013
1h pre	↑ tractament en extinció en dependència nictoina	Santa Ana et al. 2009
15- pre	= extinció en dependència cocaïna ↑ *craving*	Price et al. 2009 Price et al. 2012
1h pre	= tractament teràpia cognitiva conductual per síndrome abstinència cocaïna	Kennedy et al. 2012

Taula 1. Efectes de l'administració sistèmica o intra ABL de DCS en l'extinció i la reconsolidació de diverses tasques en animals i de l'administració sistèmica de DCS en el tractament de diverses patologies en humans [↑ millora, ↓ empitjora, = no efecte, ABL: amígdala basolateral, Acc: nucli accumbens, HPC: Hipocamp, CPL: còrtex prelímbic, CIL: còrtex infralímbic, pre: abans de l'entrenament en extinció, post: després de l'entrenament en extinció, Immed: Immediat, TEPT: trastorn per estrés posttraumàtic, TOC: trastorn obsessiu compulsiu].

b) Adquisició i consolidació de la memòria

La DCS, a part de modular els processos d'extinció i reconsolidació, regula l'adquisició i la consolidació de diferents aprenentatges en animals (Taula 2). S'ha demostrat que administrada de forma sistèmica abans de l'adquisició és capaç d'afavorir l'adquisició i la consolidació de tasques espacials (Riekkinen & Riekkinen 1997; Pussinen & Sirviö 1999; Lelong et al. 2001), del reconeixement d'objectes (Zlomuzica et al. 2007) i del condicionament clàssic (Curlik & Shors 2011). Cal afegir que també millora la consolidació de tasques d'evitació passiva (Land & Riccio 1999). D'altra banda, un menor nombre d'estudis ha avaluat els efectes de les administracions postentrenament i demostren que la DCS potencia la consolidació de la memòria en el laberint elevat (Rodgers et al. 2011; Wu et al. 2008). En relació als estudis que administren la DCS de forma intracerebral, només s'ha observat que la DCS al CPL afavoreix la retenció en una tasca de discriminació olfactòria (Villarejo-Rodriguez et al. 2010).

Estudis preclínics amb humans sans descriuen que l'administració d'aquest fàrmac té efectes beneficiosos per a la memòria. Per exemple, l'administració de DCS abans d'aprendre una tasca de memòria declarativa facilita l'adquisició, i augmenta l'activitat hipocampal, mentre que l'administració després de l'adquisició del condicionament de la por millora la seva consolidació (Kalisch et al. 2009). D'altra banda, Kuriyama et al. (2011) van demostrar que l'administració de DCS abans de l'adquisició potenciava una tasca procedimental no emocional, però que no tenia cap efecte en una tasca de memòria declarativa.

Taula 2. Efectes de la D-cicloserina en els processos d'adquisició i consolidació

MOMENT	ADMINISTRACIÓ	RESULTATS	AUTORS
Laberint aquàtic de Morris			
pre	Sistèmic	= Adquisició ↑ Activitat conductual	Pitkänen et al. 1995
		↑ Adquisició	Riekkinen et al. 1997
		↑ Adquisició = Adquisició	Lelong et al. 2001 Sunyer et al. 2008
Laberint Radial			
pre	Sistèmic	↑ Adquisició ↑ Retenció	Pussinen & Sirviö 1999
Laberint en Y			
pre post	Sistèmic	↑ Atenció ↑ lleugerament memòria	Hughes 2004
Discriminació visual			
pre	Sistèmic	↑ Adquisició	Matsuoka & Aigner 1996
Condicionament de la por			
post pre		↑ Retenció	Flood et al. 1992 Land & Riccio 1999
durant	Sistèmic	= Consolidació	Yamamoto et al. 2008
pre		↑ Adquisició tasca HPC depenent	Thompson et al. 1992
Condicionament amb menjar / aigua			
post		↑ Consolidació ↑ Recuperació	Quartermain et al. 1994
pre		↑ Adquisició = Reversió	Golden & Houpt 2007
pre	Sistèmic	↑ Adquisició	Nunnink et al. 2007 Davenport & Houpt 2009 Land & Riccio 1998
post		↑ consolidació extinció latent	Gabriele & Packard 2007

Taula 2. Efectes de l'administració sistèmica i intracerebral de DCS en l'adquisició i la consolidació de la memòria en diferents tasques en animals. (↑ millora, ↓ empitjora, = no efecte, pre: abans de l'entrenament en extinció, post: després de l'entrenament en extinció, CPL: còrtex prelímbic; HPC: Hipocamp).

1.5.2 D-cicloserina per tractar i revertir els dèficits cognitius

La utilitat de la DCS per revertir els dèficits d'aprenentatge i memòria observats en models animals de patologia neural o resultat de diverses manipulacions experimentals ha estat àmpliament estudiat (Taula 3). La majoria d'antecedents ha investigat els efectes de l'administració sistèmica de DCS i s'ha observat una millora dels dèficits cognitius associats a models d'esquizofrènia (Nishikawa 2010), de trastorn obsessiu compulsiu (Albelda et al. 2010), d'autisme (Modi & Young 2011; Otto 2010) i de la malaltia de Parkinson (Ho et al. 2011; Pawlak et al. 2012; Schneider et al. 2000). A més a més, la DCS també pot revertir els dèficits d'aprenentatge i memòria deguts a l'envelliment (Baxter

et al. 1994), la privació de son paradoxal (Silvestri & Root 2008), l'estrès (Yamamoto et al. 2008), els traumatismes cranioencefàlics (Temple & Hamm 1996), les lesions cerebrals (Riekkinen et al. 1998a,b) i les administracions de MK-801 (Matsuoka et al. 1996). Un dels models farmacològics de dèficit cognitiu més explorats en els últims anys ha estat el bloqueig de la transmissió colinèrgica amb l'administració d'SCOP (Klinkenberg & Blockland 2010) i s'ha descrit que la DCS és capaç de revertir aquests efectes (com veurem en l'apartat 2.1). Aquest fàrmac també està sent utilitzat en assajos clínics en fase II i els resultats indiquen que permet compensar dèficits deguts a la privació de son en adults sans, així com també podria ser eficient en el tractament dels símptomes, especialment els negatius, de pacients d'esquizofrènia i per millorar el processament de la informació social en els trastorns de l'aspecte autista (Kuriyama 2011; Rosse et al. 1996; Buchanan 2013; Posey et al. 2004).

D'altra banda, un menor nombre d'estudis ha avaluat els efectes de la DCS administrada intracerebralment, observant que l'administració al CPL reverteix els dèficits produïts per lesions del nucli parafascicular del tàlem (Villarejo-Rodriguez et al. 2013), a l'HPC redueix el deteriorament causat per MK-801 (Kawabe et al. 1998) i a l'amígdala compensa els dèficits induïts per l'administració de muscimol (Akirav 2007).

Taula 3. Efectes de la D-cicloserina per revertir dèficits cognitius induïts per diferents manipulacions experimentals

MANIPULACIÓ	ADMINISTRACIÓ DCS	RESULTATS	AUTORS
Laberint aquàtic de Morris			
Lesió cerebral traumàtica	Sistèmica post lesió		Temple & Hamm 1996
Lesió de l'àrea septal medial		↑ Reversió dèficit	Riekkinen et al. 1998a,b
Infusió de lidocaïna HPC dorsal	Sistèmica pre		Riekkinen et al. 1999
Exposició a toluè	Sistèmica pre	↑ Reversió dèficit ↑ rNMDA2A a l'HPC	Win-Shwe et al. 2010
Laberint radial			
Lesió HPC	Sistèmica pre		Schuster & Schmidt 1992
MK-801 Sistèmica	HPC pre	↑ Reversió dèficit	Kawabe et al. 1998
Lesions escorça entorrinal	Sistèmica pre		Zajaczkowski & Danysz 1997
Memòria de treball			
Interleuquina β a l'HPC	HPC pre		Matsumoto et al. 2001
AIDA intra-HPC	HPC pre	↑ Reversió dèficit	Ohno & Watanabe 1998
Reconeixement d'objectes			

Estrès agut	Sistèmica pre		Kart-Teke et al. 2006
Isquèmia	Sistèmica post lesió	↑ Reversió dèficit	Dhawan et al. 2011
Lesió cerebral traumàtica	Sistèmica post lesió		Yaka et al. 2007
Estrès agut	Sistèmica post		Philbert et al. 2013
Tasques de condicionament			
Muscimol Amígdala	Amígdala post	↑ Reversió dèficit	Akirav 2007
Estrès agut	Sistèmica pre	↑ Reversió dèficit en femelles ↑ Retenció mascles	Waddell et al. 2010
Privació de son REM	Sistèmica pre	↑ Reversió dèficit	Silvestri et al. 2008
Estrès postnatal	Sistèmica pre / post	↑ Reversió dèficit extinció ↑ Reversió potencials evocats en CPFm	Judo et al. 2010
Exposició endotoxina	Sistèmica post	↑ Reversió dèficit = nivells BDNF = rNMNDA1 i rNMDA2C	Kranjac et al. 2013
Knockout GluD1	Sistèmica pre	↑ reversió dèficits conducta social ↑ reversió dèficits condicionament de la por	Yadav et al. 2012 Yadav et al. 2013
Tasca de discriminació visual / olfactòria			
MK-801 Sistèmica HA-966 Sistèmica	Intramuscular pre	↑ Reversió dèficit	Matsuoka et al. 1996
Lesió PF	CPL pre		Villarejo-Rodriguez et al. 2013
Escala de severitat neurològica			
Lesió cerebral traumàtica	Sistèmica Post lesió	↑ Reversió dèficit	Adeleye et al. 2010

Taula 3. Efectes de l'administració sistèmica i intracerebral de DCS en la reversió de dèficits cognitius en diferents tasques (↑ millora, ↓ empitjora, pre: abans de l'adquisició, post: després de l'adquisició, HPC: hipocamp, PF: nucli parafasciular del tàlem, CPL: còrtex prelímbic, BDNF: Factor neurotròfic derivat del cervell).

1.5.3   D-cicloserina i plasticitat sinàptica

Amb l'objectiu d'explicar els efectes de la DCS com a potenciador dels processos d'aprenentatge i memòria, diversos estudis han investigat els mecanismes cel·lulars i moleculars subjacents a la facilitació de les funcions cognitives. S'ha observat que la DCS facilita la plasticitat sinàptica en estudis *in vitro* amb seccions d'HPC, augmenta l'amplitud dels potencials postsinàptics i facilita el manteniment de la PLT (Rouaud & Billard 2003). Aquests resultats han estat corroborats amb estudis que administren coagonistes dels rNMDA com la d-serina, la glicina o el GLYX-13, els quals troben un increment de la magnitud de la PLT (Zhang et al. 2008; Burgdorf et al. 2011; Abe et al. 1990; Henneberger et al. 2010) i un augment de la fosforilació d'ERK tant a l'HPC com a l'ABL (Matsuda et al. 2010). D'altra banda, estudis que avaluen els dèficits en la plasticitat sinàptica hipocampal deguts a manipulacions experimentals, mostren que la DCS és capaç de revertir aquest deteriorament en ratolins *knockout* per la molècula d'adhesió cel·lular neural (Kochlamazashvili et al.

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2012), en rates amb lesió cerebral traumàtica (Yaka et al. 2007), o bé sota els efectes de l'estrès (Philbert et al. 2013), així com en rates velles (Billard & Rouaud 2007; Burgdorf et al. 2011).

Els efectes facilitadors de la plasticitat sinàptica produïts per la DCS podrien ser deguts a modificacions de l'eficiència de la transmissió sinàptica derivats de canvis moleculars i reajustaments estructurals. Per exemple, la DCS modula el tràfic sinàptic dels rAMPA i augmenta la seva internalització en l'amígdala (Mao et al. 2008). D'altra banda, també s'ha observat que l'administració de DCS a l'HPC augmenta l'expressió de rNMDA2B i augmenta la neurogènesi al GD, a CA1 i a CA3 (Ren et al. 2013). La DCS quan s'administra per via sistèmica produeix un augment de l'expressió de rNMDA1A, rNMDA2A, rNMDA2B, un increment de l'activació neuronal a través de c-Fos i del marcador de plasticitat neuronal pERK al CPFm (Gupta et al. 2013), i també augmenta la supervivència neuronal a l'HPC (Curlik & Shors 2011). Addicionalment, la DCS en cultius cel·lulars augmenta la mitjana de temps i la probabilitat d'obertura del canal dels rNMDA1 i rNDMA2 comparat amb el que succeeix amb administracions de glicina (Dravid et al. 2010) i específicament sembla que el seu mecanisme d'acció té major afinitat pels rNMDA2A i rNMDA2B (Sheinin et al. 2001). Aquests resultats són congruents amb les dades que indiquen que una pèrdua específica dels rNMDA2B a l'HPC i al CPF produeix una disrupció de la plasticitat sinàptica, redueix el nombre d'espines dendrítiques i provoca dèficits d'aprenentatge (Brigman et al. 2010). A més a més, s'ha pogut observar que l'administració d'antagonistes dels rNMDA a l'HPC provoca un bloqueig de la plasticitat sinàptica (Volianskis et al. 2013), el que dóna suport igualment a les dades anteriors.

En resum, després de la revisió de la literatura podem concloure que la modulació dels rNMDA de regions cerebrals que participen en les funcions cognitives és una via essencial pel millor coneixement de les bases neurofisiològiques subjacents als processos mentals, i contribueix al desenvolupament de possibles tractaments dirigits a millorar-los.

2.   Models animals de dèficit cognitiu

A continuació tractarem, entre altres temes, com la DCS pot millorar les disfuncions trobades en diferents models de dèficit cognitiu, com són el bloqueig de la transmissió colinèrgica i el procés d'envelliment normal.

2.1   Bloqueig de la transmissió colinèrgica

L'ACh és un dels neurotransmissors més estesos al sistema nerviós, ja que les neurones colinèrgiques estan àmpliament distribuïdes per tot l'encèfal. Les principals fonts colinèrgiques són els nuclis del tronc de l'encèfal i del PB. S'ha descrit que l'ACh modula funcions cognitives i conductuals, tals com la regulació de l'arousal cortical i els processos atencionals i, a més, exerceix un paper crucial en els processos de plasticitat neural subjacents a l'aprenentatge i la memòria (Sarter & Parikh 2005; Gu 2002). Estudis farmacològics han posat de manifest que els receptors muscarínics són crítics en els processos cognitius (Bartus et al. 1982; Bhattacharya & Sen 1991). Específicament, el seu bloqueig provoca una disrupció dels processos d'aprenentatge i memòria (Hasselmo 2006), ja que l'activació d'aquests receptors redueix la permeabilitat de la membrana cel·lular al K^+ en les neurones corticals (McCormick & Prince 1985), de manera que facilita la despolarització en resposta als *inputs* excitadors glutamatèrgics. Diversos experiments han mostrat que algunes de les seves accions podrien ser regulades mitjançant la modulació dels rNMDA. En primer lloc, s'ha observat que l'ACh produeix un augment perdurable de les respostes sinàptiques de les neurones hipocampals de CA1 facilitant l'activitat dels rNMDA (Markram & Segal 1990) i, d'altra banda, s'ha involucrat en els fenòmens de potenciació i depressió a llarg termini (DLT) (Segal & Auerbach 1997; Warburton et al. 2003), i en la modulació de segons missatgers intracel·lulars (Markram & Segal 1990). Finalment, també s'ha demostrat que l'activació dels receptors muscarínics M_1 estimula l'acció de la proteïna cinasa C, facilitant així la funció dels rNMDA. En definitiva, totes aquestes accions del sistema muscarínic augmenten la probabilitat de què un estímul excitador arribi al llindar d'activació dels rNMDA, els quals modifiquen la transmissió sinàptica lligada a la plasticitat neural (Collingridge & Singer 1990).

A més dels resultats moleculars anteriors, cal afegir que s'ha observat una interacció entre els rNMDA i el sistema colinèrgic en estudis conductuals. Per exemple, el bloqueig de forma sistèmica dels rNMDA impedeix que substàncies que potencien l'activitat colinèrgica com la fisostigmina o la nicotina, per via sistèmica o a l'HPC, produeixin efectes facilitadors en la memòria implícita (Ciamei et al. 2001; Jafari-Sabet 2006). Addicionalment, s'ha demostrat que l'administració conjunta de dosis baixes de NMDA i fisostigmina, inefectives quan són administrades per separat, milloren el procés de

consolidació de la memòria emocional (Jafari-Sabet 2006). De la mateixa manera, l'administració conjunta de dosis subllindars d'antagonistes dels rNMDA i d'SCOP sistèmicament o intracerebralment deterioren l'aprenentatge i la memòria de l'evitació passiva (Ohno & Watanabe 1996; Khakpai et al 2012; Mahmoodi et al. 2010).

L'SCOP és un alcaloide que s'extreu de les plantes de la família de les solanàcies (Figura 17) i que actua com a antagonista muscarínic no selectiu, tot i que mostra una major afinitat pels receptors colinèrgics M_1 i M_5. És de caràcter competitiu, de manera que s'uneix als receptors impedint que l'ACh s'hi uneixi i pugui tenir lloc la transmissió muscarínica. L'efecte màxim es dóna 1 hora després de l'administració sistèmica i, posteriorment, va disminuint fins que desapareix al cap de 5-6 hores (Sipos et al. 1999).
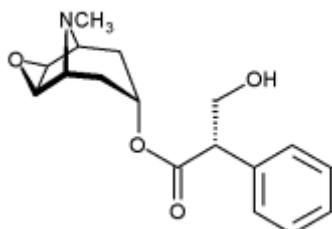
Figura 17. Composició química de l'escopolamina

Aquest fàrmac ha estat utilitzat en neuropsicofarmacologia com una droga de referència per induir dèficits cognitius, ja que reprodueix els deterioraments observats en l'envelliment o en la demència en humans sans i en animals (Ebert & Kirch 1998; Flood & Cherkin 1986). L'ús d'aquest fàrmac com a model farmacològic d'amnèsia colinèrgica prové de la hipòtesi que afirma que en el procés d'envelliment es produeix una disfunció de la memòria com a conseqüència de la disminució d'ACh (Bartus et al. 1982). Drachman & Leavitt (1974) van ser els primers en demostrar que l'administració d'SCOP a persones voluntàries sanes imitava la majoria de les disfuncions cognitives observades durant l'envelliment i la demència. Estudis posteriors en humans van demostrar, també, que el bloqueig muscarínic mitjançant l'administració d'SCOP deteriorava l'adquisició de nous aprenentatges (Atri et al. 2004) i alterava la memòria de treball (Green et al. 2005). Encara que l'administració d'antagonistes dels receptors muscarínics s'ha considerat com un model farmacològic de deteriorament cognitiu colinèrgic (Klinkenberg & Blokland 2010), l'ús de l'SCOP no està deslligat de certa controvèrsia degut al seu mode d'acció poc específic i l'ampli espectre d'efectes conductuals que indueix. Referent a això, s'ha suggerit que els antagonistes selectius de M1 podrien constituir un model farmacològic de deteriorament cognitiu relativament més vàlid, ja que són propensos a afectar la funció cognitiva d'una manera més específica (Klinkenberg & Blokland 2011).

D'altra banda, l'SCOP ha estat utilitzada en models animals per avaluar noves tasques conductuals (Ennaceur & Meliani 1992), per investigar el paper del sistema colinèrgic en la cognició i per provar la interacció amb altres fàrmacs (Levin 1988). Aquests experiments van corroborar que l'SCOP induïa dèficits en diferents paradigmes conductuals, tant administrada de forma sistèmica com intracerebral (Hodges et al. 2009; Estape & Steckler 2002; Ison & Bowen 2000; Sambeth et al. 2007). En relació a l'administració intracerebral, nombrosos estudis amb rates han permès constatar que quan s'injecta al còrtex peririnal (Abe et al. 2004; Winters & Bussey 2005), a l'HPC (Blokland et al. 1992; Riekkinen & Riekkinen 1997b; Rogers & Kesner 2003), al septe medial (Elvander et al. 2004), a l'amígdala (McIntyre et al. 1998), al còrtex insular (Miranda & Bermúdez-Rattoni 2007; Ramírez-Lugo et al. 2003), al nucli accumbens (Ramírez-Lugo et al. 2006) i al CPL (Boix-Trelis et al. 2007) produeix el deteriorament de l'aprenentatge i la memòria. Pel que fa al possible mecanisme d'acció, sembla que podria estar relacionat amb l'alteració de la plasticitat neural, ja que l'administració d'SCOP en seccions *in vitro* d'HPC provoca supressió de la PLT (Hirotsu et al. 1989; Calabresi et al. 1999; Ye et al. 2001; Sánchez et al. 2009; Lin et al. 2004), així com també deteriora la inducció de PLT hipocampal *in vivo* (Ovsepian et al. 2004).

Tenint en compte l'acció de la DCS en la PLT dependent dels rNMDA (veure punt 1.5.3), s'ha proposat que els dèficits mnemònics produïts per l'SCOP podrien ser compensats mitjançant una potenciació glutamatèrgica. Així s'ha pogut observar com l'administració sistèmica de DCS reverteix l'alteració en l'aprenentatge i la memòria produïda per l'SCOP en diferents tasques de memòria espacial, memòria de treball o discriminació d'estímuls (Taula 4). Un menor nombre d'estudis ha investigat els efectes de l'administració de DCS i SCOP a l'HPC (Ohno & Watanabe 1996; Ohno et al. 1997), en humans sans (Jones et al. 1991) i en primats no humans (Matsuoka & Aigner 1996). Tots aquests resultats donen suport, doncs, a la hipòtesi que la DCS podria ser un fàrmac adequat per pal·liar els dèficits associats a una hipofunció de les vies colinèrgiques. En el present treball, amb l'objectiu d'aprofundir en la interacció entre els rNMDA, i en concret el paper de la DCS i la transmissió muscarínica, ens centrarem en l'administració d'SCOP com a model de dèficit cognitiu.

Taula 4. Efectes de la D-cicloserina en models animals de dèficit cognitiu per administració d'escopolamina

MANIPULACIÓ SCOP	ADMINISTRACIÓ DCS	RESULTATS	AUTORS
Laberint aquàtic de Morris			
Sistèmica pre	Sistèmica pre	↑ Reversió dèficit	Sirviö et al. 1992 Puumala et al. 1998 Pitkänen et al. 1995 Fishkin et al. 1993
Laberint en T i tasca visuoespacial			
Sistèmica pre	Sistèmica pre	↑ Reversió dèficit	Fishkin et al. 1993 Pitkänen et al. 1995
Sistèmica	Sistèmica	↓ Reversió dèficit	Rupniak et al.1992
Memòria de treball			
HPC pre test	HPC pre test	↑ Reversió dèficit	Ohno & Watanabe 1996 Ohno et al. 1997 Kishi et al. 1998
Tasca de discriminació visual			
Sistèmica pre	Sistèmica pre	↑ Reversió dèficit	Andersen et al. 2002
Intramuscular pre	Intramuscular pre		Matsuoka & Aigner 1996
Evitació passiva			
Sistèmica pre	Sistèmica pre	↑ Reversió dèficit	Zajaczkowski & Danysz 1997

Taula 4. Efectes de l'administració sistèmica i intracerebral de DCS en la reversió de l'adquisició i la consolidació de la memòria en diferents tasques en animals que han rebut administracions d'escopolamina. (↑ millora, ↓ empitjora, pre: abans de l'aprenentatge).

2.2   Envelliment

Durant l'envelliment es veuen afectades, a més de la memòria, les habilitats visuoespacials i la velocitat de processament de la informació (Morse 1993). Malgrat això, variables com l'educació, el nivell d'activitat física i els factors genètics poden influir sobre les capacitats cognitives al llarg de la vida i expliquen l'elevada variabilitat existent. En funció del grau de deteriorament es pot diferenciar entre un declivi cognitiu associat a l'envelliment normal i un envelliment patològic que pot conduir a una demència (Unverzagt et al. 1998).

El procés d'envelliment normal es manifesta amb un seguit de canvis involutius al sistema nerviós central que són comuns a totes les espècies de mamífers. Aquestes alteracions de tipus morfològic que indiquen atròfia cortical, com la reducció del pes i del volum total del parènquima (Hubbard & Anderson 1981), la dilatació ventricular, l'augment de la grandària dels solcs i disminució del gruix de les circumvolucions (Terry et al. 1987), podrien explicar els dèficits cognitius que es manifesten amb major freqüència amb l'edat (Wahlund et al. 1990). Els canvis específics associats a

l'envelliment fisiològic semblen correspondre a una pèrdua selectiva de la substància blanca, mentre que una pèrdua de substància grisa de la mateixa magnitud només s'observa en condicions neuropatològiques com en la malaltia d'Alzheimer (Salat et al. 1999). A nivell microscòpic, en el cervell normal envellit apareixen freqüentment canvis neurodegeneratius com els cabdells neurofibril·lars, les plaques senils i la degeneració de les cèl·lules glials, comuns també en situacions patològiques (Morris et al. 1991; Zhang et al. 2012). La distinció entre l'envelliment normal i la demència es relaciona amb una major presència d'aquests canvis en zones rellevants per a les funcions superiors com per exemple el lòbul temporal medial, especialment l'escorça entorrinal, el lòbul temporal superior i els nuclis colinèrgics del PB (Yamamoto & Hirano 1985; Gomez-Isla et al. 1997). Finalment, des d'un punt de vista neuroquímic, es pot observar un declivi en la funció dels diferents sistemes de neurotransmissió com el dopaminèrgic, el catecolaminèrgic i el serotoninèrgic (Terry et al. 1987; Stemmelin et al. 2000), tot i que el sistema que es veu més afectat és el colinèrgic (Schliebs & Arendt 2011). En aquest sentit, els nuclis colinèrgics basals presenten fins a un 50% de pèrdua neuronal (Kemper 1993). De la mateixa manera, es produeix una gran pèrdua de cèl·lules colinèrgiques en aquests nuclis durant el desenvolupament de la malaltia d'Alzheimer (Whitehouse et al. 1982). A més, els tractaments farmacològics més utilitzats en aquesta malaltia són precisament els inhibidors de la colinesterasa, com és el cas de la tetrahidroaminoacridina, la qual sembla augmentar l'activitat colinèrgica i alleugerir la severitat de la demència en pacients d'Alzheimer (Knapp et al. 1994). D'altra banda, un altre factor que contribueix al deteriorament cognitiu observat durant l'envelliment és una reducció dels rNMDA i AMPA, els quals ja hem comentat que són essencials per l'expressió de la plasticitat sinàptica hipocampal implicada en l'aprenentatge i la memòria. En aquest sentit, concretament s'ha observat una anomalia en l'adenil ciclasa regulada pels canals de Ca^{2+} que pot implicar una reducció de la plasticitat sinàptica i del nombre de sinapsis a l'HPC (Rosenzweig & Barnes 2003). Així doncs, una de les causes principals del dèficit cognitiu associat a l'envelliment és una alteració en circuits específics de l'HPC (Nicolle et al. 1999). Un aspecte interessant és que, al contrari del que es creia anteriorment (Meaney et al. 1988), s'ha pogut comprovar com durant l'envelliment normal no s'observa una pèrdua significativa de neurones piramidals a l'HPC (Woodruff-Pak et al. 2010), però sí un empitjorament en els processos de plasticitat neural (Barnes et al. 1996), una reducció de l'arborització dendrítica (Geinisman et al. 1992) i una menor neurogènesi (Bondolfi et al. 2004).

L'envelliment del sistema nerviós té una traducció funcional molt clara, que es pot valorar en una gran quantitat de proves conductuals (Kennard & Woodruff-Pak 2011). Si bé l'execució dels animals vells és diferent de la dels animals joves en una gran varietat de tasques, també és cert que

en animals vells s'observen patrons de conducta més heterogenis. Les tasques hipocamp dependents més utilitzades en les que s'han observat dèficits han estat les proves d'aprenentatge espacial, com el LAM, (Bromley-Brits et al. 2011; Markowska et al. 1989; Rosenzweig et al. 1997), el laberint de Barnes (Barnes et al. 1980) o el laberint radial de vuit braços (Mizumori et al. 1996). De la mateixa manera, la literatura mostra que és molt comú en persones d'avançada edat tenir dificultats per recordar la relació espacial entre punts de referència i objectes (Rosenzweig et al. 2003). D'altra banda, els dèficits cognitius descrits en animals vells també afecten tasques hipocamp dependents no espacials, com la TSPA (Countryman & Gold 2007), i altres processos que no depenen essencialment de l'HPC, com la memòria de treball (Nyffeler et al. 2010) i l'aprenentatge associatiu (Yankner et al. 2008).

Tal com hem anticipat anteriorment, aquests dèficits de memòria podrien estar causats per canvis en la plasticitat sinàptica, ja que la potenciació posttetànica està disminuïda a l'HPC de rates velles (Rosenzweig & Barnes 2003). Específicament, s'observen dèficits en la inducció de la PLT quan s'utilitzen corrents d'estimulació baixes, les quals generalment desencadenen una PLT primerenca (Lynch & Voss 1994). Igualment, el manteniment de la PLT també es veu alterat, ja que en animals vells el declivi de la potenciació s'inicia abans que en animals adults (Bach et al. 1999). En congruència amb aquests resultats, les rates velles semblen ser més susceptibles a desencadenar DLT en comparació amb les rates adultes (Foster & Norris 1997). En aquest context s'ha proposat que el fet que el llindar per a la inducció de PLT sigui més alt i per a la inducció de DLT sigui menor té com a conseqüència un augment de la dificultat per codificar memòria i fa que sigui més fàcil eliminar-la (Foster 1999).

Els dèficits d'aprenentatge i memòria observats en animals vells poden ser revertits o previnguts amb determinats tractaments. S'ha demostrat que l'exercici físic pot facilitar els processos d'aprenentatge i memòria en models animals d'envelliment gràcies a una facilitació de la neurogènesi (Albeck et al. 2006). La presència d'un entorn enriquit o una dieta amb restricció calòrica també pot servir per prevenir el desenvolupament de problemes cognitius (Qiu et al. 2012; Speisman et al. 2013; Mora 2013). A més a més, les manipulacions farmacològiques que potencien l'activitat colinèrgica mitjançant agonistes nicotínics (Socci et al. 1995) o l'administració d'antagonistes GABAèrgics també permeten revertir els dèficits associats a l'envelliment (Koh et al. 2013). Malgrat tot, degut a que els mecanismes de plasticitat sinàptica depenen especialment dels rNMDA, s'ha intentat revertir els dèficits cognitius mitjançant la potenciació del sistema glutamatèrgic utilitzant, per exemple, la DCS. La majoria d'estudis amb rates velles han investigat els efectes de la DCS administrada de forma sistèmica sobre l'adquisició del LAM, observant-se en

general una millora rellevant (Riekkinen & Riekkinen 1997a; Aura et al. 1998; Baxter et al. 1994; Riekkinen et al. 1997), la qual és més potent quan es combina amb inhibidors de la colinestersasa (Aura et al. 1998). Un menor nombre d'estudis ha investigat l'administració de DCS sistèmica en altres tasques i han trobat efectes beneficiosos en el condicionament palpebral i de resposta de por (Thompson et al. 1992; Kochlamazashvili 2012). En canvi, no hem trobat cap antecedent previ que investigui els seus efectes derivats de l'administració intracerebral. Assajos clínics demostren que la DCS, conjuntament amb el tractament comú amb donepezil, millora el dèficit cognitiu en pacients amb Alzheimer (Falk et al. 2002). També s'ha observat que l'administració de DCS en aquests pacients augmenta la memòria implícita (Schwartz et al. 1996) i produeix un augment del rendiment en diverses tasques cognitives (Tsai et al. 1999).

Tal com s'ha comentat anteriorment (veure punt 1.5.3), un dels mecanismes subjacents pels quals la DCS podria estar facilitant l'aprenentatge i la memòria podria ser promovent mecanismes de plasticitat sinàptica. En aquest sentit, s'ha demostrat que els dèficits en la inducció i el manteniment de la PLT en seccions d'HPC observats en rates velles poden ser compensats per l'administració de DCS (Billard & Roudad 2007). La DCS també reverteix completament els dèficits en la PLT en ratolins vells sans i parcialment en ratolins vells *knockout* per la molècula d'adhesió cel·lular (Kochlamazashvili et al. 2012). Altres experiments han descrit que durant l'envelliment els nivells endògens de d-serina, lligand endogen del lloc d'unió de la glicina, es troben reduïts en l'HPC i l'absència d'aquest aminoàcid bloqueja la inducció de la PLT en cultius (Yang et al. 2003, Mothet et al. 2006; Junjaud et al. 2006). En conseqüència, tant l'administració de d-serina com de Glyx 13, coagonista dels rNMDA, en seccions d'HPC permet revertir els dèficits en la PLT existents en rates velles (Potier et al. 2010; Burgdorf et al. 2011).

En resum, tenint en compte els antecedents esmentats, sembla que tant en el model farmacològic d'administració d'SCOP com durant el procés d'envelliment es produeix una alteració de la transmissió colinèrgica, la qual podria explicar el deteriorament cognitiu associat. En ambdós models aquesta depleció colinèrgica podria provocar un impediment del correcte funcionament dels rNMDA i, per tant, de la plasticitat sinàptica hipocampal o d'altres regions cerebrals. Així doncs, partint d'aquesta possible interacció i relació compensatòria entre la neurotransmissió colinèrgica i glutamatèrgica, una potenciació de l'activitat dels rNMDA mitjançant l'administració de DCS podria compensar, sota certes circumstàncies, els dèficits deguts a l'administració d'SCOP i els associats a l'envelliment natural.

3. Memòria olfactòria i espacial

En el present treball, amb l'objectiu d'avaluar si la DCS és capaç de potenciar la memòria i revertir el deteriorament cognitiu associat a diferents condicions, s'han escollit dos paradigmes apetitius de memòria olfactòria, la DSO i la TSPA, i un paradigma de memòria espacial com és el LAM.

3.1 Memòria olfactòria

La memòria olfactòria ha estat considerada com un sistema únic i diferent d'altres formes de memòria (Bahuleyan & Singh 2012). Precisament l'olfacte és la modalitat sensorial que es troba físicament més propera al sistema límbic i té un caràcter molt adaptatiu, lligat a la supervivència i la reproducció. El nivell més alt de processament té lloc a l'escorça olfactòria peririnal, la qual està estretament relacionada amb l'amígdala, l'HPC i regions del CPF. S'ha demostrat que un deteriorament en el reconeixement, la identificació i el llindar de detecció de les olors està associat amb la demència i les malalties neurodegeneratives (Bahuleyan & Singh 2012). Concretament, en les malalties d'Alzheimer i de Parkinson s'ha trobat un empitjorament primerenc significatiu de les funcions olfactòries (McNamara et al. 2008) i per aquest motiu s'ha suggerit que la detecció d'anòsmia en la fase preclínica podria ser utilitzada com a indicador durant el diagnòstic diferencial d'aquestes malalties (Solomon 1994; Doty et al. 1987; Daniel & Hawkes 1992).

3.1.1. Discriminació simple d'olors

La DSO és un aprenentatge associatiu simple que utilitza senyals olfactoris i es basa en la tendència natural i innata dels animals a explorar preferentment els estímuls nous i discriminar entre els estímuls coneguts. És, per tant, una tasca apetitiva naturalista i de ràpida adquisició, la qual permet un record consistent que es manté a llarg termini (Sara et al. 1999). Aquesta tasca va ser desenvolupada per Susan Sara et al. (1999) amb l'objectiu d'estudiar la dinàmica temporal de diferents receptors en la formació de la memòria a llarg termini, i evitar possibles estímuls aversius que poguessin intervenir en la cascada d'AMPc-proteïna cinasa A implicada en el manteniment de la PLT (Stanton & Sarvey 1985). Aquesta tasca consisteix a discriminar tres aromes diferents impregnades en tres esponges idèntiques i associar una d'aquestes olors amb un reforç. Una de les tres aromes és l'EC, inicialment neutre, el qual s'associa amb l'EI, un reforç de caràcter apetitiu. Després de successives associacions entre l'EC i l'EI, l'aroma reforçada (EC+) acaba adquirint un caràcter apetitiu per a l'animal i provoca un augment en la preferència del subjecte per a dirigir-se a l'esponja que conté l'aroma reforçada.

Aquesta tasca es realitza en un espai quadrat tancat on es col·loquen les tres esponges (Figura 18). La fase d'adquisició consisteix en una única sessió de diversos assajos (entre 3-5), en els que els animals se situen a la cantonada de la caixa sense esponja com a punt de sortida i, entre assaig i assaig, es canvia la configuració espacial de les esponges amb l'objectiu d'evitar que el subjecte es pugui guiar per pistes contextuals, però reforçant sempre la mateixa esponja. A més, el fet de reforçar sempre la mateixa aroma obliga al subjecte a inhibir la seva tendència innata a explorar estímuls novadors, és a dir, les altres aromes, i buscar sempre la mateixa esponja aromatitzada per a obtenir el reforç (Sara et al. 1999). Per tal d'avaluar el nivell d'aprenentatge es mesuren dues variables, la latència dels subjectes en emetre la resposta correcte, és a dir, introduir el musell dins de l'esponja reforçada i consumir el cereal i, en segon lloc, el nombre d'errors comesos. Els errors poden ser introduir el musell dins d'una esponja no reforçada (errors d'olor), o bé olorar l'esponja reforçada i no introduir-hi el musell (error d'omissió) (Figura 18). A continuació, per tal d'avaluar la memòria olfactòria, es duu a terme el test de retenció de la DSO en una sola sessió idèntica a la d'adquisició, on es mesura el record, la resistència a l'extinció i la capacitat de reaprenentatge de la tasca.
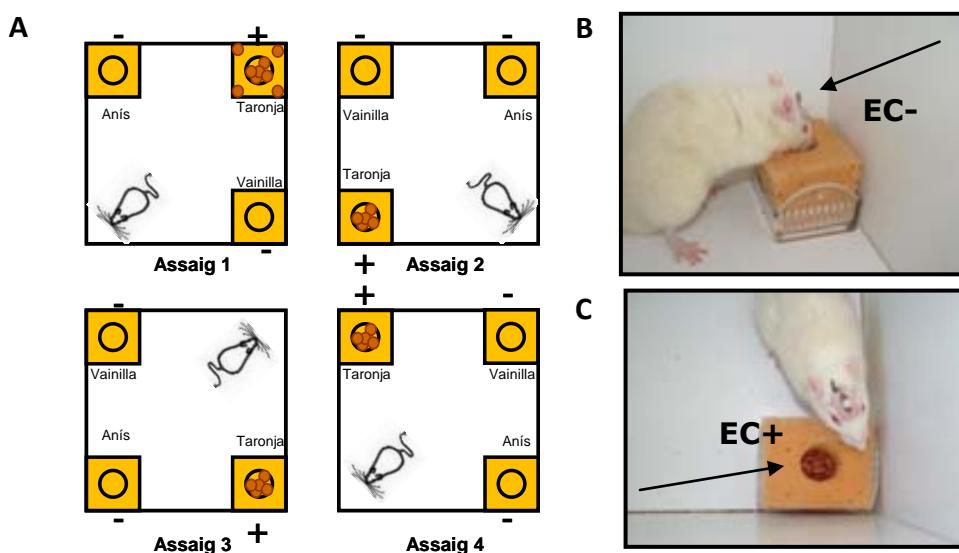
Figura 18. (A) Esquema gràfic de la sessió d'adquisició de la tasca DSO. Representació dels dos tipus d'errors enregistrats: (B) error d'olor (C) error d'omissió.

Per tal d'identificar el substrat neuroanatòmic i neuroquímic de la DSO (Annex 7), els primers estudis van investigar els efectes de la injecció de diferents substàncies en el sistema ventricular. Els resultats van demostrar que els rNMDA intervenien en les primeres fases de la consolidació, ja que el seu bloqueig immediatament després de l'adquisició (Tronel & Sara 2003) i de la reactivació de la memòria (Torras-Garcia et al. 2005) deterioraven els processos de consolidació i reconsolidació. En canvi els receptors adrenèrgics participaven en fases més tardanes, ja que el seu bloqueig només

provocava alteració de la memòria quan l'administració es realitzava després de l'aprenentatge (Sara et al. 1999). Les regions cerebrals més implicades amb l'adquisició i consolidació d'aquesta tasca són el CPL, especialment durant l'adquisició, (Kublik & Sara 2003), el còrtex orbitofrontal ventrolateral i l'ABL (Tronel & Sara 2002). En relació a la neuroquímica, els rNMDA i els receptors muscarínics del CPL participen en la primera fase de consolidació de la memòria de la DSO, ja que el seu bloqueig produeix dèficits de memòria importants (Tronel & Sara 2003; Carballo-Marquez et al. 2007), mentre que l'administració de DCS facilita la retenció i compensa el dèficit de les lesions del nucli parafascicular del tàlem, que envia projeccions glutamatèrgiques al CPL (Villarejo-Rodriguez 2010; Villarejo-Rodriguez 2013).

En resum, el paradigma de DSO permet avaluar la capacitat dels animals per diferenciar estímuls olfactoris i identificar un estímul reforçant. Aquest aprenentatge associatiu procedimental sembla dependre principalment dels circuits dels quals formen part el CPL i l'ABL, però no l'HPC (Tronel & Sara 2003).

3.1.2. Transmissió social de preferència alimentària

La TSPA és un altre paradigma apetitiu de memòria olfactòria, que a més de dependre del CPL i l'ABL, també requereix la integritat de l'HPC pel seu record. Aquesta tasca va ser desenvolupada per primera vegada, de manera simultània i independent, pels equips de Bennett G. Galef i Posadas-Andrews l'any 1983 (Galef & Wigmore 1983; Posadas-Andrews & Roper 1983). La TSPA ocorre de manera espontània a la natura quan un animal observador interacciona amb un membre de la mateixa espècie, animal demostrador, que acaba de consumir un aliment i, aleshores, l'animal observador prefereix el mateix aliment que ha ingerit l'animal demostrador abans que un aliment desconegut. Aquesta interacció i transmissió de la informació i la posterior preferència alimentària els permet disminuir el risc de menjar aliments nous que podrien no ser segurs (Galef & Wigmore 1983).

En condicions de laboratori, la TSPA ens permet estudiar els mecanismes cerebrals que conformen la memòria relacional naturalista. Durant la interacció entre els animals, l'observador forma una associació entre l'olor de l'aliment aromatitzat i un component natural i volàtil de l'alè del demostrador, el disulfur de carboni (CS_2) (Galef et al. 1988). Posteriorment a aquest aprenentatge social, els subjectes observadors són sotmesos a un test de preferència alimentària en el que trien entre dos aliments aromatitzats diferents, un dels quals és el que ha consumit anteriorment l'animal demostrador. Si els subjectes observadors han après bé l'associació entre els dos estímuls, en el test de preferència consumiran una major quantitat de l'aliment amb l'aroma que prèviament havia ingerit el subjecte demostrador, respecte a l'altre aliment també aromatitzat però desconegut (Galef et al.

1985) (Figura 19). Així doncs, els subjectes observadors han de fer un ús flexible de la informació olfactòria adquirida, ja que només un dels dos estímuls amb què han format l'associació durant l'aprenentatge, l'aroma de l'aliment, és present en el test de preferència alimentària per a poder guiar l'execució de la seva conducta (Alvarez et al. 2001; Bunsey & Eichenbaum 1995). Altres aspectes que porten a identificar la TSPA com a un model de memòria relacional són que la informació és apresa ràpidament en un únic episodi i que l'expressió de la memòria es duu a terme en una situació (selecció de menjar) molt diferent del context en què es dóna l'aprenentatge (interacció social).

Els primers estudis van demostrar que els animals eren capaços d'adquirir aquest aprenentatge gràcies a la codificació dels senyals olfactoris emesos pels demostradors. Per aquesta raó, quan els animals observadors es trobaven separats per una paret transparent que no permetia el pas de les olors o bé se'ls bloquejava el sentit de l'olfacte, aquests no eren capaços de desenvolupar la preferència per l'aliment prèviament ingerit pel demostrador (Galef & Wigmore 1983). No obstant això, aquest aprenentatge no era resultat d'una simple exposició a l'aroma sinó que era necessària l'olor associada a l'aliment més el CS_2 de l'alè de la rata demostradora (Galef et al. 1985). És a dir, quan els observadors oloraven només un aliment no desenvolupaven cap preferència, mentre que si oloraven l'aliment dipositat en una rata anestesiada o bé un cotó fluix impregnat per una olor més CS_2 diluït, sí que mostraven un correcte aprenentatge (Galef et al. 1985; Galef et al. 1988; Burne et al. 2010). Per tant, aquests canvis en les preferències dels subjectes eren produïts per l'exposició de l'animal en un context social de transmissió d'informació.
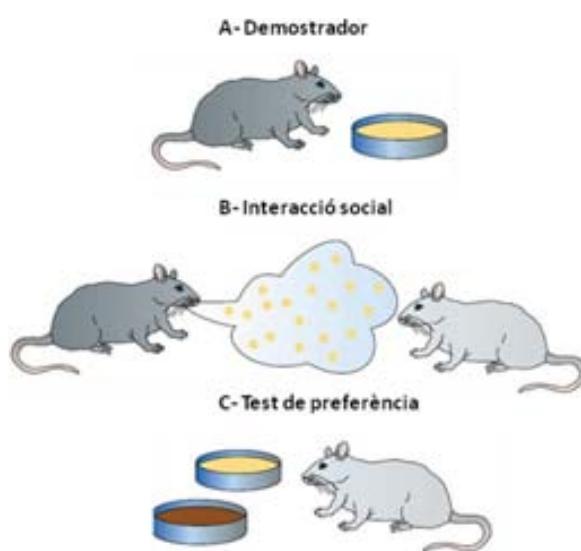

Figura 19. Tasca de transmissió social de preferència alimentària. A) L'animal demostrador consumeix un aliment aromatitzat. B) Interacció social entre el demostrador i l'observador. C) Test de preferència alimentària on es mesura l'aprenentatge de l'observador (Adaptat de Eichenbaum 2000).

Aquesta tasca és suficientment robusta com per no veure's afectada per altres factors determinants en l'elecció de la dieta, malgrat que s'han de tenir en compte algunes variables que podrien modular l'aprenentatge. Per exemple, si hi ha molta diferència entre l'atractiu pel sabor entre les dues aromes a escollir hi haurà un menor efecte del context social en l'elecció (Galef & Whiskin 1998). També té un efecte el grau de familiaritat o l'experiència amb un aliment, ja que s'aprèn millor la preferència per una dieta no familiar que per una dieta familiar (Galef 1993; Galef & Whiskin 1994). Sembla, per tant, que els animals utilitzen la informació social principalment per seleccionar nous aliments i expandir el seu repertori alimentari amb aliments segurs (Forkman 1991; Galef & Whiskin 1994; Murtton 1971). D'altra banda, la preferència per la dieta apresa es perllonga d'una manera duradora i estable en el temps (Galef 1989; Galef & Whiskin 2003), ja que els animals la poden recordar fins a tres mesos després de l'aprenentatge (Clark et al. 2002), tot i rebre informació d'altres aliments durant aquest interval (Galef et al. 2005).

En rates, la influència social en la selecció de la dieta és extraordinàriament intensa i és independent de variables com el sexe, l'edat, la privació d'aliment, el tipus d'aliment utilitzat, el nivell de coneixença entre els animals o la soca de rata (Galef et al. 1984). No obstant, s'ha observat que les rates velles presenten una pèrdua prematura del record de la TSPA en comparació amb rates més joves (Countryman & Gold 2007). A més, si bé l'espècie més estudiada ha estat la rata (Galef 2005), aquest aprenentatge s'ha observat també en altres espècies de rosegadors (Valsecchi & Galef 1989; Galef et al. 1998; Solomon et al. 2002; Lupfer et al. 2003), així com s'ha establert en moltes altres espècies, com humans, aus i altres mamífers (Birch 1980; Mason et al. 1984; Lupfer-Johnson & Ross 2007).

L'estructura cerebral que més s'ha investigat en relació a la TSPA, ha estat l'HPC degut a què es tracta d'una tasca de tipus relacional. En general, les lesions electrolítiques de la formació hipocampal, tant de la part ventral com de la part dorsal, prèvies a l'adquisició de la TSPA produeixen dèficits de la memòria a llarg termini sense una afectació de l'aprenentatge inicial (Winocur 1990; Winocur et al. 2001), els quals correlacionen amb l'extensió de l'afectació de l'HPC (Alvarez et al. 2001; Bunsey & Eichenbaum 1995; Clark et al. 2002). Les lesions produïdes immediatament després de l'entrenament, tant a l'HPCd com a l'HPCv, ocasionen una amnèsia retrògrada gradual, és a dir, una pèrdua de memòria dels fets més recents previs a la lesió. Aquests dèficits no s'observen quan la lesió es produeix molts dies després de l'adquisició (Winocur 1990; Winocur et al. 2001; Clark et al. 2002; Ross & Eichenbaum 2006). En relació als neurotransmissors i mecanismes moleculars implicats en l'execució de la TSPA, s'ha observat que la transmissió glutamatèrgica és crucial per a aquesta memòria social olfactòria. Així doncs, l'administració d'antagonistes dels rNMDA abans de l'adquisició,

directament a CA1, o de forma sistèmica, impedeixen la retenció de la TSPA, però, si s'administren després de l'adquisició no tenen cap efecte (Roberts & Shapiro 2002; Burne et al. 2010). Així mateix, ratolins *knockout* dels NMDAR1 de CA1 mostren dèficits en la consolidació de la memòria (Rampon & Tsien 2000). A més a més, també s'ha demostrat rellevant l'activitat colinèrgica durant l'adquisició de la TSPA, doncs s'ha observat un augment de l'alliberació d'ACh a l'HPC durant la interacció social (Gold et al. 2011). Addicionalment, s'ha posat de manifest que la sobrexpressió tant al l'HPCd com a l'HPCv d'una mutació de CREB o bé la inhibició de l'expressió de c-Fos deterioren la memòria a llarg termini de la TSPA (Brightwell et al. 2005; Countryman et al. 2005a). Convé ressaltar que s'ha trobat un augment de l'expressió de c-Fos i de la fosforilació de CREB a l'HPC després de l'adquisició i el record de la TSPA (Countryman et al. 2005b), que és especialment marcat a l'HPCv durant el test de memòria (Countryman & Gold, 2007; Smith et al. 2007). Altres estudis corroboren el deteriorament de la memòria a llarg termini de la TSPA en ratolins que no expressen Thy1, una molècula d'adhesió cel·lular que quan és absent provoca una excessiva inhibició GABAèrgica específicament al GD (Mayeux-Portas et al. 2000), o bé en ratolins que no expressen la subunitat $\alpha(1B)$ dels canals de Ca^{2+} dependents de voltatge de les regions hipocampals CA3 i CA1 (Jeon et al. 2007). Altres manipulacions que també incoluen l'HPC han descrit dèficits en la memòria de la TSPA en ratolins *knockout* de mGluR1 (Kishimoto et al. 2002), en ratolins *knockout* de la proteïna P311, implicada en la transformació i la motilitat de les cèl·lules neurals, (Taylor et al. 2008) i en alteracions de la subunitat Kvb1.1 dels canals de K^+ tipus A (Giese et al. 1998).

A més de la formació hipocampal, per a la correcta execució de la TSPA s'ha descrit la participació d'altres regions cerebrals. En primer lloc, diversos estudis han investigat el paper del PB, ja que s'ha evidenciat que el sistema colinèrgic central està involucrat en el processament de senyals olfactoris i en la formació i el record de memòries de reconeixement social (Ravel et al. 1994; Winslow & Camacho 1995). En conseqüència, les lesions colinèrgiques específiques preentrenament, utilitzant la immunotoxina colinèrgica 192 immunoglobulina G saporina, tant de la part més rostral (SM/BDBv) com de la part més caudal (NBM/SI), produeixen un deteriorament de la retenció de la TSPA, sense afectar la memòria immediata (Berger-Sweeney et al. 2000). En canvi, altres estudis observen un impediment en la retenció tant a curt com a llarg termini amb les lesions específiques preentrenament del NBM/SI i un dèficit en la consolidació de la memòria amb les lesions del SM/BDBv (Vale-Martínez et al. 2002). Sembla, doncs, que l'NBM és especialment crític en l'aprenentatge d'aquesta tasca, i aquest resultat es veu corroborat pel fet que la seva estimulació elèctrica provoca una millora de l'adquisició i de la retenció de la TSPA (Boix-Trelis et al. 2006). En aquesta regió també s'ha avaluat el rol de la galanina, ja que la seva expressió en el PB augmenta notablement en la malaltia d'Alzheimer

(Bowser et al. 1997), i en ratolins transgènics provoca una sobrexpressió que impedeix la memòria de la TSPA. En segon lloc, l'amígdala també sembla tenir una certa rellevància en la TSPA, segurament perquè rep els principals *inputs* olfactoris des de l'escorça olfactòria piriforme (Pare 2003). Amb tot, els resultats mostren una certa contradicció, ja que en general suggereixen que l'ABL és necessària per a l'adquisició de la TSPA però no per a la seva retenció a llarg termini (Wang et al. 2006). Específicament, el bloqueig muscarínic amb SCOP de l'ABL abans de l'adquisició provoca dèficit en la retenció de la tasca (Carballo-Marquez et al. 2009b). En tercer lloc, l'escorça frontal també participa en la TSPA degut a què les lesions, tant pre com postentrenament, afecten el test de retenció quan s'introdueix una tercera opció de resposta i el dèficit augmenta quan major és l'interval de temps entre l'entrenament i el test (Winocur & Moscovitch 1999). Altres treballs han demostrat que el bloqueig colinèrgic del còrtex orbitofrontal (Ross et al. 2005) o del CPL (Boix-Trelis et al. 2007; Carballo-Marquez et al. 2009a) produeix dèficit de memòria en diferents tests de retenció. A més, s'ha observat un patró d'activació de c-Fos important en regions prefrontals, tant després de l'adquisició de la TSPA com després de la seva recuperació (Smith et al. 2007). En quart lloc, el diencèfal també sembla estar implicat en la TSPA, ja que les lesions hipotalàmiques postentrenament afecten el record de la TSPA (Winocur 1990). A més, lesions preentrenament del nucli talàmic parafascicular provoquen dèficits en la retenció de la TSPA (Quiroz-Padilla et al. 2006), el què suggereix que aquest nucli podria modular l'adquisició de la TSPA probablement a través de les seves connexions amb el còrtex CPF.

En resum, la TSPA, a més d'ésser una tasca olfactòria i social de tipus naturalista, és un paradigma no espacial que permet estudiar la memòria relacional. Entre les estructures cerebrals que hi participen, l'HPC ha estat la que ha rebut una major rellevància en especial per la consolidació de la memòria, mentre que les altres estructures com el PB, l'amígdala, el CPF i el diencèfal semblen contribuir més a l'adquisició de la tasca.

3.2 Memòria espacial

L'aprenentatge espacial ha estat avaluat àmpliament en els darrers anys i és un dels primers processos cognitius que es veuen alterats en diverses malalties neurodegeneratives com la malaltia d'Alzheimer (Vaughan et al. 2006). Un dels instruments més utilitzats en l'avaluació de les característiques i els mecanismes neuronals de la memòria espacial ha estat el LAM.

3.2.1 Laberint aquàtic de Morris

El LAM va ser desenvolupat per Richard G.M. Morris el 1981, amb l'objectiu d'estudiar l'aprenentatge espacial i la funció de les cèl·lules de lloc, i ràpidament es va estendre popularitzant-

se el seu ús en la comunitat neurocientífica (Kolb et al. 1982). Tot i que sovint es parla del LAM com una tasca *per se* és més aviat un instrument en el qual es poden dur a terme múltiples tasques. Específicament, el LAM consisteix en una piscina circular plena d'aigua en la qual els animals, generalment rates o ratolins, han de nadar fins a trobar una plataforma lleugerament submergida sobre la que acaben situant-se per evitar seguir nedant (Figura 20). La temperatura de l'aigua oscil·la entre 18 i 22ºC, el que fa que els animals vulguin escapar sense un augment del nivell d'estrès tan elevat com per provocar una inhibició de la conducta de recerca de la plataforma. L'aprenentatge es mesura a partir de la latència de fugida, és a dir, el temps que l'animal triga a trobar la plataforma en cadascun dels assajos de l'entrenament. Així, les latències curtes s'interpreten com a un correcte aprenentatge. Per tal de trobar la localització de la plataforma els animals solen disposar d'un entorn ric en referències ambientals, anomenades pistes espacials (llums, caixes, joguines, etc.), les quals ajuden a orientar la seva conducta natatòria. En funció del disseny utilitzat es podrà modificar aquest entorn per tal de realitzar des de tasques simples a protocols d'entrenament complexes que impliquen mecanismes diferents de navegació, aprenentatge i memòria. La seva sensibilitat a diferents manipulacions experimentals ha fet que els paradigmes d'entrenament possibles en el LAM siguin inclosos en bateries de proves conductuals com a eina per avaluar l'impacte de diferents alteracions del sistema nerviós com ara dany cerebral, envelliment, neurodegeneració, o substàncies terapèutiques.
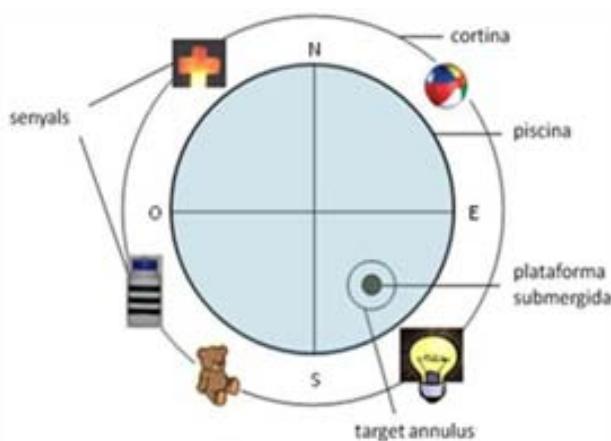
Figura 20. Representació gràfica del laberint aquàtic de Morris

El protocol bàsic d'aprenentatge és el que avalua memòria de referència i consisteix en col·locar una plataforma amagada, en posició fixa respecte a les pistes de l'habitació i introduir els animals a l'aigua des de diferents punts d'entrada. En la fase d'adquisició es deixa nedar l'animal lliurament per tal que busqui la plataforma basant-se en les pistes externes. En el cas de no trobar-la en el màxim de temps per assaig, l'animal és guiat manualment per l'experimentador fins a la

plataforma. Aquest procediment es repeteix en els diferents assajos i sessions d'entrenament, el nombre dels quals pot variar en funció de l'exigència del protocol i el tipus de subjecte. Després de la fase d'adquisició es realitza un assaig de prova o test de retenció en el que es retira la plataforma de la piscina i si l'animal ha après a localitzar la plataforma d'acord amb les pistes distals al laberint mostrarà una trajectòria centrada en l'emplaçament previ de la plataforma. S'ha descrit que una disminució de la latència de fugida pot ser deguda a l'ús per part de l'animal d'estratègies no espacials, com nedar en cercles concèntrics a certa distància de la paret fins a trobar la plataforma (Maei et al. 2009). Per tant, amb l'objectiu de poder discriminar entre estratègies espacials i no espacials, generalment es duu a terme un assaig de prova que permet comprovar si en absència de la plataforma, les rates exploren durant un percentatge de temps més elevat el quadrant del LAM on hauria d'estar la plataforma. El protocol bàsic d'entrenament pot incloure també una sessió de reversió addicional, en la que la plataforma es traslllada a un quadrant diferent del de l'aprenentatge original sense modificar l'escenari general de la piscina. Aquesta prova ens informa de l'habilitat de generalització i flexibilitat que l'animal ha adquirit amb l'entrenament precedent per realitzar aquest tipus d'aprenentatge (Wenk 2004). Durant els assajos d'adquisició, de prova i els de reversió, la mesura d'aprenentatge més utilitzada, com s'ha mencionat prèviament, és la latència de fugida dels animals, ja que és una variable poc sensible als canvis ambientals (Crabbe 1999), no obstant també es valoren altres variables com la velocitat de la conducta natatòria, la distància transcorreguda i la conducta de tigmotaxis com a mesures de control (Maei et al. 2009).

Anatòmicament, el sistema hipocampal és el més implicat en l'aprenentatge i la memòria de la informació espacial i contextual. Aquest sistema participa en la formació d'associacions o representacions complexes entre estímuls (Eichenbaum et al. 1999; D'Hooge & De Deyn 2001), ja que integra funcionalment múltiples àrees corticals rellevants per a la percepció i la memòria i intervé en la codificació de les associacions entre les característiques espacials i temporals dels estímuls ambientals (Daselaar et al. 2006; Kessels et al. 2001). Diversos estudis amb animals han demostrat que les lesions de diferents estructures de la formació hipocampal causen deteriorament en l'execució del LAM (Terry 2009; Morris 1984; Poucet et al. 2000), així com les lesions hipocampals en humans també solen produir dèficits greus d'orientació espacial (Astur et al. 2002). Tot i que tradicionalment s'ha descrit que les lesions de l'HPCd produeixen un major deteriorament en l'aprenentatge del LAM que les de l'HPCv (Moser et al. 1993), estudis recents han descrit que la regió dorsal és crucial per a la conducta espacial i la regió ventral per la codificació del context (Nadel et al. 2013) i en ambdues regions s'han trobat cèl·lules de lloc (Moser et al. 2008) (veure apartat 1.3.1).

D'altra banda, s'ha observat que algunes regions de l'escorça cerebral també poden estar implicades en l'aprenentatge espacial. Per exemple, s'han descrit cèl·lules a l'escorça parietal amb

propietats complementàries a les cèl·lules de lloc de l'HPC. La seva activació sembla relacionar-se amb l'orientació del cap respecte a l'entorn, independentment de la localització, i, per tant, en el processament de senyals proximals (Cressant et al. 1997). A més, s'ha observat, que les rates amb lesions prefrontals presenten dèficits en els processos de planificació, els quals impedeixen la formació d'una adequada representació de la seqüència de moviments necessària per trobar la plataforma (Granon & Poucet 1995). Finalment, s'ha demostrat que lesions del nucli accumbens i del cerebel deterioren els aspectes procedimentals de l'execució del LAM (Ploeger et al. 1994; D'Hooge & De Deyn 2001).

S'han estudiat diferents sistemes de neurotransmissió relacionats amb l'aprenentatge i la memòria espacial, però el Glu i l'ACh han estat els que han rebut una major atenció (Myhrer 2003). Així doncs, les manipulacions farmacològiques mitjançant antagonistes colinèrgics o glutamatèrgics afecten negativament l'adquisició del LAM (Rison & Stanton 1995; Wesierska et al. 1990). Específicament, s'ha demostrat que els rNMDA de l'HPC són essencials per aquesta tasca perquè modulen diferents aspectes de l'adquisició i la retenció (Woodside et al. 2004) i el seu bloqueig impedeix l'adquisició del LAM i paral·lelament altera la PLT (Morris 1984). Estudis previs han demostrat que la potenciació de l'activitat glutamatèrgica pot produir una facilitació de l'aprenentatge espacial, ja que l'administració sistèmica de coagonistes dels rNMDA, com la DCS, accelera aquest aprenentatge (Taula 5). Finalment, com que ambdós sistemes de neurotransmissió poden trobar-se alterats durant l'envelliment, essent l'HPC una de les regions més afectades, és lògic observar alteracions en l'execució del LAM en rates velles (Gallagher & Nicolle 1993; Gallagher et al. 1993; Gallagher et al. 2003).

Taula 5. Efectes de la D-cicloserina sistèmica preentrenament en el laberint aquàtic de Morris

SUBJECTES	RESULTATS	ESTUDI
Rates adultes tractades amb SCOP		Sirvio et al. 1992
		Fishkin et al. 1993
		Pitkänen et al. 1995
		Puumala et al. 1998
		Baxter et al. 1994
Rates velles (24 mesos)	↑ Reverteix dèficits	Riekkinen & Riekkinen 1997a
		Riekkinen et al. 1997
		Aura et al. 1998
Rates amb lesió traumàtica		Temple & Hamm 1996
Rates amb lesió area septal medial		Riekkinen et al. 1998a,b
Rates amb lesió HPC dorsal	↓ reverteix	Riekkinen et al. 1999
Rates velles (24 mesos)	= retenció	Aura et al. 2000
	↑ adquisició	Aura et al. 2000
Rata adulta	=	Sunyer et al. 2008

Taula 5. Efectes de diferents manipulacions experimentals sobre l'adquisició i la retenció del laberint aquàtic de Morris. [↓: disminueix; ↑: augmenta; =: no afecta]

IV. Experimental Research

IV. Experimental Research

1.   Experiments 1 and 2: Efectes de la DCS a l'ABL en l'extinció i la reconsolidació de la DSO

D-cycloserine in the basolateral amygdala prevents extinction and enhances reconsolidation of odor-reward associative learning in rats.

Portero-Tresserra M, Martí-Nicolovius M, Guillazo-Blanch G, Boadas-Vaello P, Vale-Martínez A.

1.1   Theoretical approach

It has been shown in several studies the importance of N-Methyl D-Aspartate receptors (NMDAR) and the participation of the basolateral amygdala (BLA) in olfactory memory. It is also widely accepted that extinction and reconsolidation are NMDAR-dependent processes and also that the BLA is a critical brain region for such cognitive processes. Former results demonstrated that systemic or intra-BLA administration of D-cycloserine (DCS), a partial agonist at the glycine recognition site in the NMDAR, enhanced extinction and reconsolidation in fear conditioning paradigms. However, there is no agreement about the effects of DCS on extinction of appetitive tasks and experiments using natural rewards, such as food, have been designed to a lesser degree. The present study is based on the hypothesis that the modulation of NDMAR in the BLA may be critical for the extinction and reconsolidation of an appetitive olfactory discrimination task (ODT) and, consequently, two experiments were conducted:

- Experiment 1: The effects of the administration of DCS into the BLA on the ODT extinction were evaluated through the infusion of DCS 20 minutes before the extinction learning. Twenty four hours after the extinction, a memory test measuring extinction retention was performed and, following that, an additional reacquisition session was included as an indirect measure of extinction.
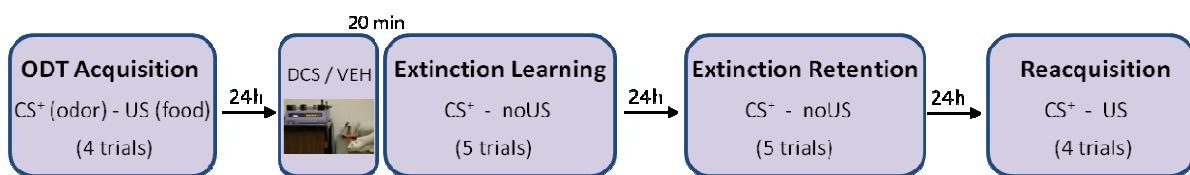
- Experiment 2: The role of the infusion of DCS into the BLA on the ODT reconsolidation was assessed. Similarly to the previous experiment, DCS was infused 20 minutes before the reactivation session and tested 24 hours later.
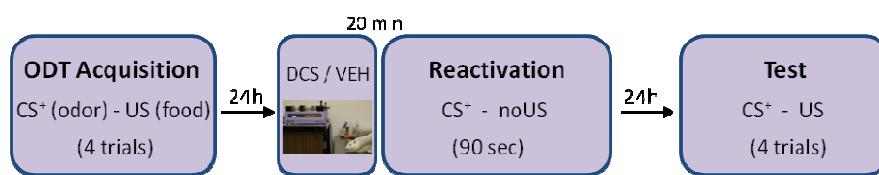
1.2 Experiment 1

1.2.1 Methods and procedure (Figure 21)

The aim of this experiment was to explore the effects of the infusion of DCS into the BLA in ODT extinction learning and retention. Hence, rats were trained in the ODT task and 20 minutes prior to the extinction learning they received bilateral intra-BLA infusions of DCS (10µg/side) or VEH. Twenty four hours later, a free-drug extinction retention test was carried out and the next day a reacquisition session was performed. The dose was determined on the basis of previous research (Villarejo-Rodriguez et al. 2010) and earlier literature relating to intra-BLA infusions of DCS (Mao et al. 2006).

To carry out this experiment, the final sample was made up of 18 Wistar rats which were distributed into two experimental groups: DCS (n=9) and VEH (n=9). At the beginning of the experiment, all the subjects were habituated to the training box and the food reinforcement (crispy chocolate cereal) and they underwent sterotaxic implantation of bilateral chronic guide cannulae in the BLA. After the surgery, the rats were allowed to recover for one week and then they were submitted to another habituation session and also adapted to a mock infusion protocol. After seven days, the ODT acquisition session (4 trials) was carried out. Three sponges were aromatized with three different odors (anise, vanilla and orange) and placed in any of the three corners of the box. The reinforcement was associated with the same odor across trials, and the target odor was randomly assigned to each rat. Latency before a correct response (nose poke into the reinforced sponge) and number of errors were scored. Two different kinds of errors were combined: errors of commission (nose poke into the incorrect sponges) and omissions (failure to nose poke after sniffing the sponge containing the target odor). Twenty four hours after the ODT acquisition, the animals were gently immobilized and microinjectors were introduced through the cannulae guides (Plastics One) to administrate the substances. The control group received PBS (phosphate-buffered saline 0.1 M PH 7.4) and the experimental group a dose of 10µg of DCS (Sigma-Aldrich) dissolved in PBS. The solution was infused bilaterally in a volume of 0.5 µl/hemisphere for 2 minutes and a rate of 0.25µl/min. After 20 minutes, the extinction learning was performed in a single 5-trial session. The procedure was the same than in the acquisition but no food reward was placed in any of the sponges for any of the trials. The variables recorded were the same, but long latencies and more number of errors were indexes of good extinction learning and it was also established an extinction criterion (latency ≥44 s to make the correct response). Twenty four hours after the extinction learning, a free-drug retention test, with the same conditions than the previous session, was included. Finally, 24

hours after the extinction retention, a reacquisition session was performed. This session was similar to the acquisition session and the original rewarded odor was again associated with the cereal. At the end of the experiment, an additional olfactory perception test was conducted to rule out olfactory impairments due to the DCS administration. Twenty minutes before such test, the animals received infusions of DCS or PBS and then they were placed in a cage, where a piece of a sweet-smelling cookie was buried, and the latency to find it was recorded.
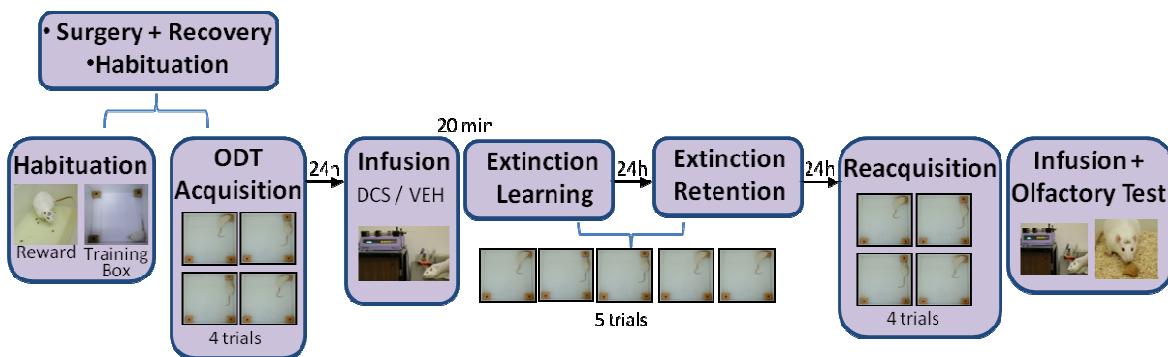
Figure 21. The behavioral procedure used in the experiment 1

1.2.2. Summary of the main results

- The NMDAR co-agonist DCS administered into the BLA prior to the ODT extinction learning disrupted both learning and retention as the group receiving DCS showed shorter latencies and fewer errors during both extinction sessions. Thus, DCS and VEH animals began the extinction learning from a similar level of performance but diverged across subsequent trials. Moreover, the DCS group needed a higher number of trials to achieve the extinction criterion than the VEH group.

- The resistance observed in extinction learning and retention in the DCS group seemed to facilitate the ODT reacquisition session. In this case, the DCS rats also showed shorter latencies and fewer errors than VEH rats.

- The DCS infusion did not produce any effect on olfactory perception since all the animals exhibited similar latencies to find a buried cookie.

1.3   Experiment 2

1.3.1   Methods and procedure (Figure 22)

In this experiment the effects of DCS infusions into the BLA were analyzed on ODT reconsolidation. Thus, rats were trained in the ODT task and 24 hours following acquisition the reactivation session was carried out in which two groups of animals followed a reactivation protocol and two groups did not. Twenty minutes prior to the memory reactivation, all groups received a bilateral intra-BLA infusion of DCS (10μg/side) or VEH. Twenty four hours later the animals performed a drug-free reconsolidation memory test.

To carry out this experiment, the final sample was made up of 46 Wistar rats which were divided into four experimental groups: DCS-REACT (n=12), VEH-REACT (n=12), DCS-nonREACT (n=12), VEH-nonREACT (n=10). In this experiment, the behavioral procedures of habituation, surgery and acquisition were the same than those described for experiment 1, but the rats underwent a reactivation session 24 hours after the acquisition consisting of a brief 90 seconds exposure to a small sponge infused with the original reinforced odor without including food reward. Twenty minutes prior to the reactivation, the rats received a bilateral intra-BLA infusion of DCS, or PBS and 24 hours after the reactivation session, they were tested in a 4-trial reconsolidation test session, using the same procedure as in the acquisition. Finally, to rule out olfactory impairments due to the DCS administration, an olfactory perception test was conducted 24 hours after the animals received infusions of DCS or PBS, as it was described in the experiment 1.
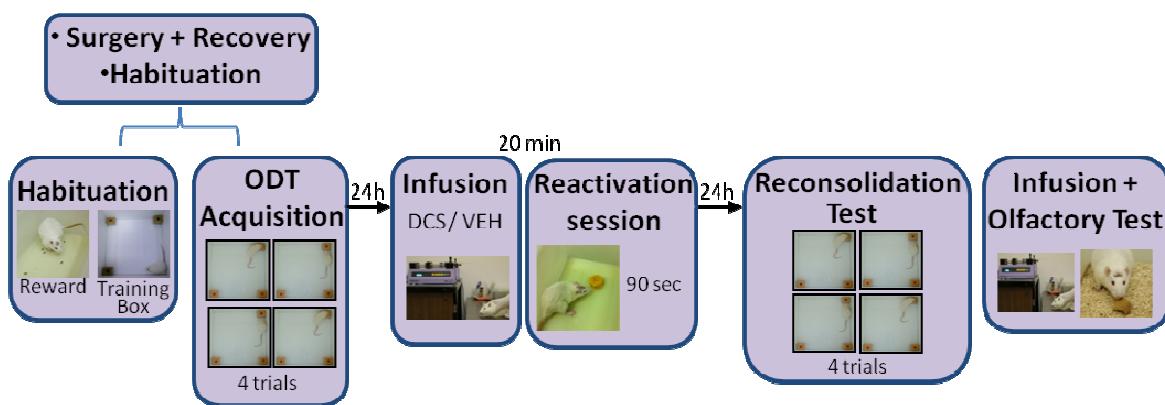
Figure 22. The behavioral procedure used in the experiment 2

1.3.2 Summary of the main results

- The administration of DCS into the BLA prior to memory reactivation enhanced ODT memory reconsolidation in a subsequent 24-hour retention test. The group reactivated receiving DCS displayed shorter latencies and less number of errors during the retention session than the VEH group.

- The mere reexposure to the odor or the DCS injection alone did not notably enhance the ODT memory. The interaction between both factors, drug and reactivation, seems, therefore, necessary to enhance reconsolidation.

- The DCS has not affected the olfactory perception since all groups performed similarly in the olfactory test.

D-cycloserine in the basolateral amygdala prevents extinction and enhances reconsolidation of odor-reward associative learning in rats

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ABSTRACT

It is well established that D-cycloserine (DCS), a partial agonist of the NMDA receptor glycine site, enhances learning and memory processes. Although the effects of DCS have been especially elucidated in the extinction and reconsolidation of aversive behavioral paradigms or drug-related behaviors, they have not been clearly determined in appetitive tasks using natural reinforcers. The current study examined the effects of pre-retrieval intra-basolateral amygdala (BLA) infusions of DCS on the extinction and reconsolidation of an appetitive odor discrimination task. Rats were trained to discriminate between three odors, one of which was associated with a palatable food reward, and, 20 min prior to extinction learning (experiment 1) or reactivation (experiment 2), they received bilateral intra-BLA infusions of DCS or vehicle. In experiment 1, DCS infusion reduced the rate of extinction learning, weakened extinction retention in a post-extinction test and enhanced reacquisition of the ODT task. In experiment 2, DCS improved subsequent memory expression in the reconsolidation test performed one day after the reactivation session. Such results indicate the involvement of BLA NMDA receptors in odor-food reward associative memory and suggest that DCS may potentiate the persistence or strength of the original memory trace.

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1. Introduction

NMDA-receptor transmission is involved in learning processes through its role in the modulation of glutamatergic activity. More specifically, NMDA receptor (NMDAR) agonists have been regarded as pharmacological treatments that enhance learning, and as such, have been considered potential cognitive enhancers (see Villarejo-Rodríguez, Vale-Martínez, Guillazo-Blanch, & Martí-Nicolovius, 2010). D-cycloserine (DCS) is a partial agonist at the glycine recognition site of the NMDAR and has been shown to enhance acquisition, consolidation, relearning, extinction and reconsolidation in several associative learning paradigms (Bustos, Giachero, Maldonado, & Molina, 2010; Curlik & Shors, 2011; Davis, 2002; Ledgerwood, Richardson, & Cranney, 2005; Lee, Milton, & Everitt, 2006; Rodgers, Harvest, Hassall, & Kaddour, 2011; Villarejo-Rodriguez et al., 2010). The majority of studies have focused on the role of DCS in extinction and reconsolidation, as the modulation of such processes may be useful as a treatment strategy for maladaptive memory or anxiety disorders (Davis, Ressler, Rothbaum, & Richardson, 2006;

Hofmann, 2007). Although extinction and reconsolidation are both triggered by memory retrieval, reconsolidation is thought to reinforce or update the expression of the original memory while extinction weakens it, possibly through the formation of a new memory trace involving an inhibitory learning process (de la Fuente, Freudenthal, & Romano, 2011; Duvarci, Mamou, & Nader, 2006; Kindt, Soeter, & Vervliet, 2009). An important determinant of subsequent memory expression following reactivation is the length of the reminder experience with brief cues initiating reconsolidation but longer cues resulting in memory extinction (Pedreira & Maldonado, 2003; Suzuki et al., 2004; Tronson & Taylor, 2007).

Recent research has reported the role of DCS in the extinction of learned behavior involving appetitive stimuli, such as drug-seeking behavior, suggesting that systemic administration of DCS facilitates the extinction consolidation of self-administration and conditioned place preference (CPP) associated to different drugs (Botreau, Paolone, & Stewart, 2006; Kelley, Anderson, & Itzhak, 2007; Nic Dhonnchadha et al., 2010; Paolone, Botreau, & Stewart, 2009; Thanos, Bermeo, Wang, & Volkow, 2009, 2011) and enhances the persistence of extinction (Groblewski, Lattal, & Cunningham, 2009). In contrast to such reports, a recent study showed that pre-treatment with systemic DCS prior to extinction training had no effect on the extinction and subsequent reinstatement of morphine-induced CPP (Lu, Wu, Zhang, Ai, & Li, 2011). Food-motivated tasks

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have been studied to a lesser degree and also indicate inconsistent results. Although systemic injections of DCS enhanced extinction consolidation of operant behavior (Leslie, Norwood, Kennedy, Begley, & Shaw, 2012; Shaw et al., 2009) and latent extinction consolidation in a food-reward maze (Gabriele & Packard, 2007), it slowed down the extinction learning of an appetitive instrumental food-rewarded response (Port & Seybold, 1998). Also, more recent studies have reported the same treatment to have no effect on the extinction learning of an appetitive operant response reinforced with food (Flavell, Barber, & Lee, 2011; Vurbic, Gold, & Bouton, 2011) or on extinction and spontaneous recovery of conditioned taste aversion (Mickley et al., 2012). Such discrepancies may depend on the timing of DCS administration, as the studies using pre-training injections were predominant in showing impairments or no effects on extinction (Lu et al., 2011; Port & Seybold, 1998; Vurbic et al., 2011) in contrast to post-extinction administration (Gabriele & Packard, 2007; Leslie et al., 2012; Shaw et al., 2009). Similarly, there are several reports from human studies suggesting that administration of DCS prior to extinction has no effect on fear conditioning (Klumpers et al., 2012), exposure therapy for post-traumatic stress disorder (Litz et al., 2012) or alcohol dependence (Watson et al., 2011), and that it enhances craving in the case of cocaine (Price et al., 2012) and alcohol dependence (Hofmann, Hüweler, MacKillop, & Kantak, 2012).

The basolateral amygdala (BLA) is a brain region that has received a great deal of attention in terms of memory extinction and reconsolidation, and especially so in aversive paradigms (Lee et al., 2006; Nader, Schafe, & Le Doux, 2000; Rehberg, Bergado-Acosta, Koch, & Stork, 2010). Previous literature has mainly examined the effects of DCS on contextual or auditory conditioned fear, indicating that systemic and intra-BLA administrations of DCS may potentiate both extinction and reconsolidation (Ledgerwood, Richardson, & Cranney, 2003; Ledgerwood et al., 2005; Lee et al., 2006; Toth et al., 2012; Walker, Ressler, Lu, & Davis, 2002; Yamada, Zushida, Wada, & Sekiguchi, 2009; Yamamoto et al., 2008). Also, DCS in the BLA facilitated the extinction of contextual fear with no effect on the extinction of conditioned taste aversion (Akirav, Segev, Motanis, & Maroun, 2009). However, there has been very little systematic investigation as to the intra-BLA effects of DCS in the extinction and reconsolidation of appetitive paradigms, particularly those using natural incentives. Although there are no reports on the effects of pre-training DCS infusions into the BLA on extinction, there are some studies using post-training infusions that show divergent results. While Torregrossa, Sanchez, and Taylor (2010) found no effect in the extinction of cocaine-associated cues, Botreau et al. (2006) reported the facilitation of cocaine-induced CPP extinction formation. As for reconsolidation studies, intra-BLA infusions prior to reactivation facilitated memory reconsolidation of stimulus-cocaine association in a self-administration paradigm (Lee, Gardner, Butler, & Everitt, 2009).

To determine whether extinction and reconsolidation of an appetitive model is modulated by pre-retrieval intra-BLA DCS, we used a simple food reward-based olfactory paradigm. This odor discrimination task (ODT) involves a rapidly acquired association between odor and palatable food reward and allows for consistent memory. It entails neither fear nor acute stress and is sensitive to NMDA receptors (NMDARs) manipulation (Tronel & Sara, 2003). Thus, infusions of NMDARs antagonists in the prelimbic cortex (PLC) have been shown to prevent initial ODT consolidation (Tronel & Sara, 2003) and disrupt reconsolidation when infused into the cerebral ventricles (Torras-Garcia, Lelong, Tronel, & Sara, 2005). In contrast, pretraining DCS infusions in the PLC enhance relearning without affecting initial acquisition or consolidation (Villarejo-Rodriguez et al., 2010) and ameliorate lesion-induced ODT deficits (Villarejo-Rodriguez, Boadas-Baello, Portero, Vale-Martinez, Marti-Nicolovius & Guillazo-Blanch, 2012). Although, as far as we know,

there is no previous evidence that NMDARs in the amygdala are involved in ODT, the BLA has been related to this task as it is markedly activated after acquisition, but not after retrieval, of the odor-reward association (Tronel & Sara, 2002). Nevertheless, as stated above, although the BLA is critically involved in extinction and reconsolidation of a variety of tasks, its specific role in such processes in ODT has not yet been determined.

Therefore, the present study is the first to explore the role of BLA NMDARs activation in ODT extinction and reconsolidation. For this purpose, the agonist DCS was injected directly into the BLA (10 µg/site) 20 min prior to extinction learning (experiment 1) or memory reactivation (experiment 2) of the odor-reward association. In experiment 1, a reacquisition session was carried out after extinction training to obtain an indirect measure of the animals' extinction level. The first experiment involved re-exposing rats to the conditioned stimulus (CS, rewarded odor) in the absence of the unconditioned stimulus (US, chocolate cereal) during 5 consecutive trials in two extinction sessions (learning and retention). As DCS was infused just before the extinction learning, in which the first trial may act as a trigger reminder leading to a reconsolidation process, the second experiment was designed to test whether the DCS was able to improve reconsolidation per se after a brief exposure to CS (90-s of rewarded odor) also in the absence of US. Dose and drug administration moment were based on previous results suggesting extinction, reconsolidation and relearning facilitation (see 2.3).

2. Material and methods

2.1. Subjects

Seventy-two male Wistar rats belonging to our laboratory's breeding stock were used: 22 rats in experiment 1 (mean age = 91d, SD = 5.02; mean weight = 393.1 g, SD = 27.4 at the beginning of the experiment) and 50 in experiment 2 (mean age = 92.8d, SD = 8.9; mean weight = 380.20 g, SD = 41.0). All the rats were single-housed in $50 \times 22 \times 14$ cm plastic-bottomed, sawdust-bedded cages in a room controlled for temperature (20–22 °C) and humidity (40–70%). The rats were maintained on a 12 h light–dark cycle (lights on at 8:00 a.m.), with experiments performed during the light phase of the cycle. Rat-chow pellets (Scientific Animal Food & Engineering, Augy, France) and water were provided ad libitum with the exception of habituation, acquisition, extinction, reacquisition and reconsolidation test sessions, in which the rats were submitted to a food restriction schedule (12 g/day) to maintain their body weight at 85% of their free-feeding weight. The animals were handled on a daily basis for 5 min and restrained for 2 min to accustom them to the injection procedure. All procedures were carried out in compliance with the European Community Council Directive for care and use of laboratory animals (86/609/ECC) and with the Generalitat de Catalunya's authorization (DOGC 2450 7/8/1997, DARP protocol number 5959).

2.2. Surgery

Animals were anesthetized with isoflurane and underwent stereotaxic implantation of bilateral chronic guide cannulae in the BLA following procedures explained elsewhere (Carballo-Marquez, Vale-Martinez, Guillazo-Blanch, & Marti-Nicolovius, 2009). Each guide cannula comprised one 26-gauge metal tube projecting 7.5 mm from the pedestal (Plastics One®, Bilaney Consultants GMBH, Germany). The stereotaxic coordinates used for implantation into the BLA were: AP: −2.6 mm from bregma; ML: ±4.9 mm from midline, and DV: −7.5 mm from cranium surface (Paxinos & Watson, 1997). Sterile dummy stylets (Plastics One®) were placed

into the cannulae to prevent occlusion. After surgery, the rats were replaced in their home cages for seven days prior to behavioral training (3 days for recovery, 3 days for food restriction, and 1 day for habituation). During the 7-day recovery period, the rats were handled and weighed on a daily basis and the dummy stylets were changed every other day.

2.3. Microinfusion procedure

Twenty minutes prior to the ODT extinction learning (experiment 1) or reactivation (experiment 2) sessions, the rats were gently restrained while the dummy stylets were removed and replaced with a 33-gauge stainless-steel injector (Plastics One®) extending 1 mm below the cannula tips. The injection-to-extinction learning/reactivation interval was the same as in previous research showing a positive effect of DCS on ODT performance (Villarejo-Rodriguez et al., 2010) and similar to that used in other experiments (15–30 min) reporting facilitation of intra-BLA DCS on extinction (Lee et al., 2006; Mao, Hsiao, & Gean, 2006; Walker et al., 2002) and reconsolidation (Lee et al., 2009; Yamada et al., 2009). The injectors were connected by polyethylene tubing (Plastics One®) to two 10-μl syringes (SGE Analytical Science, Cromlab S.L. Barcelona, Spain) mounted in an infusion pump (11 Plus Syringe Pump, Harvard Apparatus Inc., USA). DCS (Sigma–Aldrich, Madrid, Spain) was dissolved in PBS (phosphate-buffered saline 0.1 M pH 7.4) and a dose of 10 μg/hemisphere was infused in the DCS groups. The solution was infused bilaterally in a volume of 0.5 μl/hemisphere for 2 min. The dose was also determined both on the basis of our previous research with ODT (Villarejo-Rodriguez et al., 2010) and previous literature relating to intra BLA infusions (Ledgerwood et al., 2003; Mao et al., 2006). The inner cannulae were left in place for 1 min after the infusion was complete to allow for diffusion. The rats in the VEH groups received PBS injections under the same conditions.

2.4. Apparatus

The training apparatus and behavioral procedures are explained elsewhere (Carballo-Marquez et al., 2007). The training apparatus was a $60 \times 60 \times 40$ cm square box containing three sponges ($8.5 \times 6.5 \times 5.5$ cm) with a 3-cm diameter hole cut into the center to a depth of 2.5 cm, placed in glass slide-holders of the same size. The food reinforcement used was a crispy chocolate rice breakfast cereal (Kellogg's, Spain) that was placed at the bottom of the opening in the sponge. Each sponge was infused with an odor that was injected into each of its corners. The odors, vanilla (0.3 ml), orange (0.6 ml) and anise (0.2 ml) (Vahiné, Ducros S.A., Sabadell, Spain), were previously tested in a pilot study in which the rats showed no particular preference. All behavioral sessions were recorded by a video camera (JVC, Everio Model GZ-X900) connected to a monitor.

2.5. Behavioral procedures in experiment 1: ODT extinction

2.5.1. Habituation sessions

The rats were food-deprived for five days prior to three habituation sessions in which they were given free access to the reinforcement in a plastic bottomed cage ($50 \times 22 \times 14$ cm). After consuming ten pieces of cereal, they were placed in the training box, without the reinforcement, and allowed to explore it for 15 min. Once the rats had recovered from surgery, they were once again food-deprived and submitted to an identical habituation session. On the same day, the rats were also adapted to a mock infusion protocol (no solutions injected) in order to minimize any stress associated with the procedure.

2.5.2. Acquisition session

One day after the post-surgery habituation, ODT acquisition was carried out in a 4-trial session (see Fig. 2A), in accordance with previously described procedures (Quiroz-Padilla, Guillazo-Blanch, Vale-Martinez, Torras-Garcia, & Marti-Nicolovius, 2007). The reinforcement (US, chocolate rice cereal placed at the bottom of the opening in the target sponge) was associated with the same odor across trials (CS⁺), and the rewarded odor was randomly assigned to each rat in a counterbalanced manner. The sponges with the non-rewarded odors did not contain any food. Sponges were placed in any three of the four corners of the box, and the position of each odor within the box was changed for each trial according to a previously determined protocol. The rats were placed in the training box, facing the corner without a sponge. After the rats had found and eaten the cereal, they remained in the box for a few seconds before being removed and placed in the intertrial cage for 1 min before the following trial. There was a 3-min maximum period for the rat to find and consume the reinforcement. Failure to find and eat the cereal within this period resulted in the rat's being removed from the training box and placed in the intertrial cage for 1 min before the following trial. The rats with latencies longer than three min in each acquisition trial were excluded from the analyses on the grounds that they had not correctly learned the task (n = 2). Latency before a correct response (nose-poking into the rewarded sponge) and number of errors were scored as dependent variables. Two different errors were combined: errors of commission (nose-poking into a non-rewarded sponge) and omissions (sniffing the rewarded sponge with no subsequent nose-poking) (Tronel & Sara, 2003).

2.5.3. Extinction learning

Twenty-four hours after the acquisition session, the rats received a bilateral intracerebral infusion of DCS (DCS group) or PBS (VEH group) in the BLA 20 min prior to ODT extinction learning. Extinction learning was carried out in a single 5-trial session as a pilot study indicated that five trials allowed an optimum extinction of the odor-reward association. The procedure used was the same as in the preceding acquisition session, but no food reward was placed in any of the sponges for any of the trials (5 trials, CS⁺/no US). The trials were considered to be complete when the subjects had made the conditioned response, nose-poking into the previously rewarded sponge (CS⁺), or after three min. The variables recorded were the same as in the previous session, but a large number of errors and long latencies to nose-poke into the formerly rewarded sponge were an index of good extinction learning. An extinction criterion was also established: latency, during 2 consecutive trials, to make the formerly correct response ⩾44 s, which was the mean latency observed in the first trial of the acquisition session (when the animals had not yet learned the task).

2.5.4. Extinction retention

Twenty-four hours after the extinction learning session, a drug-free test (extinction retention) similar to the previous session was carried out. The rats were once again placed in the training box and underwent five further extinction trials without reward (5 trials, CS⁺/no US).

2.5.5. Reacquisition session

Twenty-four hours after extinction retention, a final session, in which the rats were returned to the original learning conditions, was performed. In this session, the rewarded odor was again associated with the cereal, as in the acquisition session (4 trials, CS⁺/US).

2.6. Behavioral procedures in experiment 2: ODT reconsolidation

Behavioral procedures of habituation and acquisition were similar to those described for experiment 1.

2.6.1. Reactivation session

Twenty-four hours after acquisition, the rats received a reactivation session (see Fig. 4A) consisting of a brief exposure (90 s) to a small sponge (different from the one used in the ODT training) infused with the reinforced odor, within the inter-trial cage, in the same room the acquisition had taken place. No food reward was present during this session, in which motor activity and contacts with the sponge were directly observed by the experimenter. Four experimental groups were studied, but only two received the memory reactivation (REACT groups) while the other two remained in a quiet room adjacent to the experimental room (nonREACT groups). Twenty minutes prior to the reactivation protocol, the rats from both groups received a bilateral intraBLA infusion of DCS (DCS REACT and DCS nonREACT) or PBS (VEH REACT and VEH nonREACT).

2.6.2. Test session

Twenty-four hours after the reactivation session, rats were tested in a 4-trial retention/reconsolidation session using the same procedure as in acquisition. The originally rewarded odor was again associated with the cereal with the exception that the first trial was not reinforced to measure memory of the previous training (see also Torras-Garcia et al., 2005).

2.7. Olfactory perception test

To rule out olfactory impairments due to the infusion of DCS, an additional olfactory perception test was conducted at the end of each experiment (Carballo-Marquez et al., 2007; Quiroz-Padilla, Guillazo-Blanch, Vale-Martinez, & Marti-Nicolovius, 2006; Wrenn, Harris, Saavedra, & Crawley, 2003). Twenty-four hours prior to the olfactory test, the rats were habituated to butter-flavored cookies (Brambly Hedge, Denmark). They were then food-restricted for 24 h prior to the infusion and the test. Twenty minutes (experiment 1) or 24 h (experiment 2) before the test, they were infused with DCS (10 µg) or PBS. The test was conducted in clean rat cages ($50 \times 22 \times 14$ cm) and a piece of cookie was buried in one corner of the cage. The rats were then placed in the cage, and the latency to find the buried cookie and commence eating was timed.

2.8. Histology

Upon completion of the behavioral study, the rats were deeply anesthetized with an overdose of sodium pentobarbital (Dolethal, 200 mg/kg; Vetoquinol S.A., Madrid, Spain) and perfused transcardially with PBS (pH: 7.4) followed by 4% paraformaldheyde in 0.1 M PBS at a flow rate of 40 ml/min. Subsequently, the cannulae were carefully removed and brains were postfixed in paraformaldehyde for 2 h and then submerged in a 20% sucrose solution prior to sectioning. Coronal 40-µm sections were cut on a cryostat (Shandom Cryotome FSE, Thermo Electron Corporation, Massachusetts, USA), mounted and processed for acetylcholinesterase histochemistry, essentially as described elsewhere (Paxinos & Watson, 1997). The sections were examined to verify cannula placement by two independent observers under a light microscope (Olympus BX 41; Olympus Optical CO, LTD, Japan). Microphotographs of the cannula placements were obtained using a digital camera (Olympus DP70).

2.9. Data analysis

In experiment 1, data were submitted to a mixed analysis of variance using repeated measures (ANOVA; PASW v19) in which the between-factor was group (DCS and VEH) and the within-factor was session. The session factor consisted of 4 measures: acquisition (the average scores for the 4 trials), extinction learning (the average scores for the 5 trials), extinction retention (the average scores for the 5 trials) and reacquisition (the average scores for the 4 trials). The dependent variables were latencies and number of errors. Any deviations from sphericity were corrected using the Greenhouse-Geisser correction if $p < 0.05$, and corresponding contrasts were performed when necessary. Trial-by trial analyses of the latencies and errors during the acquisition session, extinction learning, extinction retention and reacquisition sessions (18 trials) were performed by means of ANOVA. An additional survival analysis (procedure Kaplan–Meier and Breslow contrasts) was carried out to analyse and compare the mean number of extinction trials, during learning and retention sessions, required by each experimental group in order to reach the pre-established extinction criterion (see Section 2.5.3).

In experiment 2, analyses of variance were carried out, with session as a within-subject factor that consisted of 2 measures: acquisition (the average scores for the 4 trials) and test (the average scores for the 4 trials), and drug (DCS and VEH) and reactivation (reactivated vs. non-reactivated) as the between-subject factors following a 2×2 design. The dependent variables measured were latencies and errors. Additionally, a trial-by trial analysis of latencies and errors during the test session was performed by means of ANOVA with the group factor consisting of 4 categories (DCS-REACT, DCS-nonREACT, VEH-REACT, VEH-nonREACT). Bonferroni correction was used for multiple comparisons.

Regarding the olfactory perception test, two additional ANOVA analyses (injection-test delay 20 min and 24 h) were applied considering group (DCS and VEH) as the independent variable and latency in finding the buried cookie as the dependent variable.

3. Results

3.1. Histology (experiments 1 and 2)

Only rats with patent microinjector tips within the boundaries of the BLA were included in the analyses. Subjects were only included if their injector tips were located bilaterally within the BLA (in the area delimited by the basomedial amygdala, the lateral amygdala, the basolateral posterior amygdala and the bed nucleus of the stria terminalis) and no tissue damage, caused by the rate or volume of infusions, was detected (Fig. 1A). Specifically, the cannulae were located along different brain coordinates from 2.30 mm to 3.14 mm posterior to bregma (Paxinos & Watson, 1997) (Fig. 1B and C). Subjects with incorrectly implanted cannulae (located in other amygdalar nuclei) were excluded from the analyses (rats in experiment 1: DCS, $n = 2$; rats in experiment 2: DCS-REACT, $n = 2$; DCS-nonREACT, $n = 1$; VEH-REACT, $n = 1$).

3.2. Behavior

3.2.1. Experiment 1: ODT extinction

The DCS group ($n = 9$) showed significant disruption of ODT extinction learning and memory when compared to the VEH group ($n = 9$), demonstrated by shorter latencies and a lower number of errors. The analysis of the latencies as an average for all trials in each session (Fig. 2B) showed statistically significant differences between both groups ($F_{(1,16)} = 11.424$, $p = 0.004$). There were also significant effects of the session factor ($F_{(3,48)} = 18.764$, $p < 0.001$)
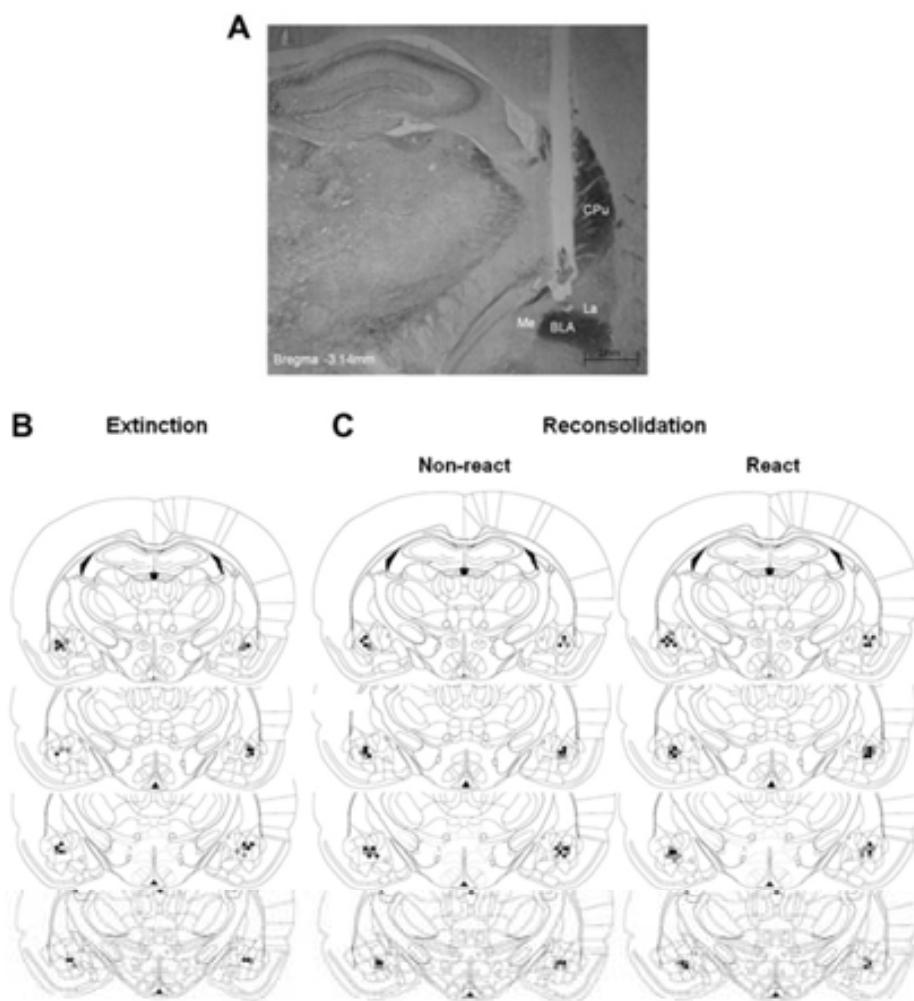
Fig. 1. (A) Photomicrograph (2 × magnification) of acetylcholinesterase staining at the level of BLA (AP, 3.14 mm posterior to bregma) showing the cannula track and the microinjector tip of a representative subject [BLA, basolateral amygdala; CPu, caudate-putamen; La, lateral amygdala; Me, medial amygdala]. (B, C) Location of injectors within the BLA. Schematic representation of the brain at four rostro-caudal levels (□2.30, □2.56, □2.80 and □3.14 mm from bregma). PBS-infused rats are represented by empty circles and DCS-infused rats by filled circles, for experiment 1(B) and experiment 2 (C).

and the group × session interaction ($F_{(3,48)} = 6.512$, $p = 0.004$), indicating that behavior across the four sessions was different between both groups. Specifically, both groups showed a similar learning level in the acquisition session ($F_{(1,16)} = 0.265$, $p = 0.614$), but DCS rats showed shorter latencies in extinction learning ($F_{(1,16)} = 15.627$, $p = 0.001$), extinction retention ($F_{(1,16)} = 12.067$, $p = 0.003$) and reacquisition ($F_{(1,16)} = 4.912$, $p = 0.042$). Moreover, a within-group analysis showed that the DCS group did not show any difference between the acquisition session and the remaining sessions. In contrast, statistically significant differences were found in the VEH group between the acquisition session and extinction learning ($p < 0.001$), extinction retention ($p < 0.001$) and reacquisition sessions ($p = 0.024$). All these results suggested that only VEH rats exhibited clear signs of extinction that may have slowed down subsequent reacquisition.

The trial-by-trial analysis confirmed statistically significant effects of group ($F_{(1,16)} = 12.340$, $p = 0.003$), trial ($F_{(17,272)} = 12.112$, $p < 0.001$) and group × trial interaction ($F_{(17,272)} = 3.283$, $p < 0.001$). As can be seen in Fig. 2C, both groups began from a similar level, with no differences in any of the acquisition trials or the first extinction trial, and they progressively differed throughout extinction learning (trial 2: $F_{(1,16)} = 7.022$, $p = 0.017$; trial 4: $F_{(1,16)} = 11.572$, $p = 0.004$), extinction retention (trial 1: $F_{(1,16)} = $

4.723, $p = 0.045$; trial 3: $F_{(1,16)} = 5.355$, $p = 0.034$; trial 4: $F_{(1,16)} = 15.056$, $p = 0.001$ and trial 5: $F_{(1,16)} = 9.137$, $p = 0.008$) and reacquisition (trial 1: $F_{(1,16)} = 6.051$, $p = 0.026$).

Such results were completed by a survival analysis of the latencies within both extinction sessions (Fig. 3), which indicated that the DCS rats were slower to extinguish their behavior as they needed more trials to reach the extinction criterion (see Section 2.5.3), and also the percentage of DCS subjects achieving the criterion was significantly lower than VEH subjects in extinction learning ($X^2 = 8.837$, df = 1, $p = 0.003$) and extinction retention ($X^2 = 4.537$, df = 1, $p = 0.033$). Thus, by the end of the extinction learning, 90% of the animals in the VEH group reached the criterion vs. 55.5% of rats in the DCS group, and by the final retention extinction trial, 100% of the VEH rats achieved the criterion vs. only 66.6% of the rats injected with DCS.

The analysis of the number of errors (Fig. 2D) showed statistically significant between-group differences ($F_{(1,16)} = 14.613$, $p = 0.001$), but the session ($F_{(3,48)} = 0.499$, $p = 0.685$) and the session × group interaction ($F_{(3,48)} = 2.028$, $p = 0.122$) were not statistically significant. The between-group differences were particularly evident in commission errors ($F_{(1,16)} = 12.622$, $p = 0.003$) as opposed to omission errors ($F_{(1,16)} = 3.609$, $p = 0.076$). The trial-by-trial analysis (Fig. 2E) showed that the
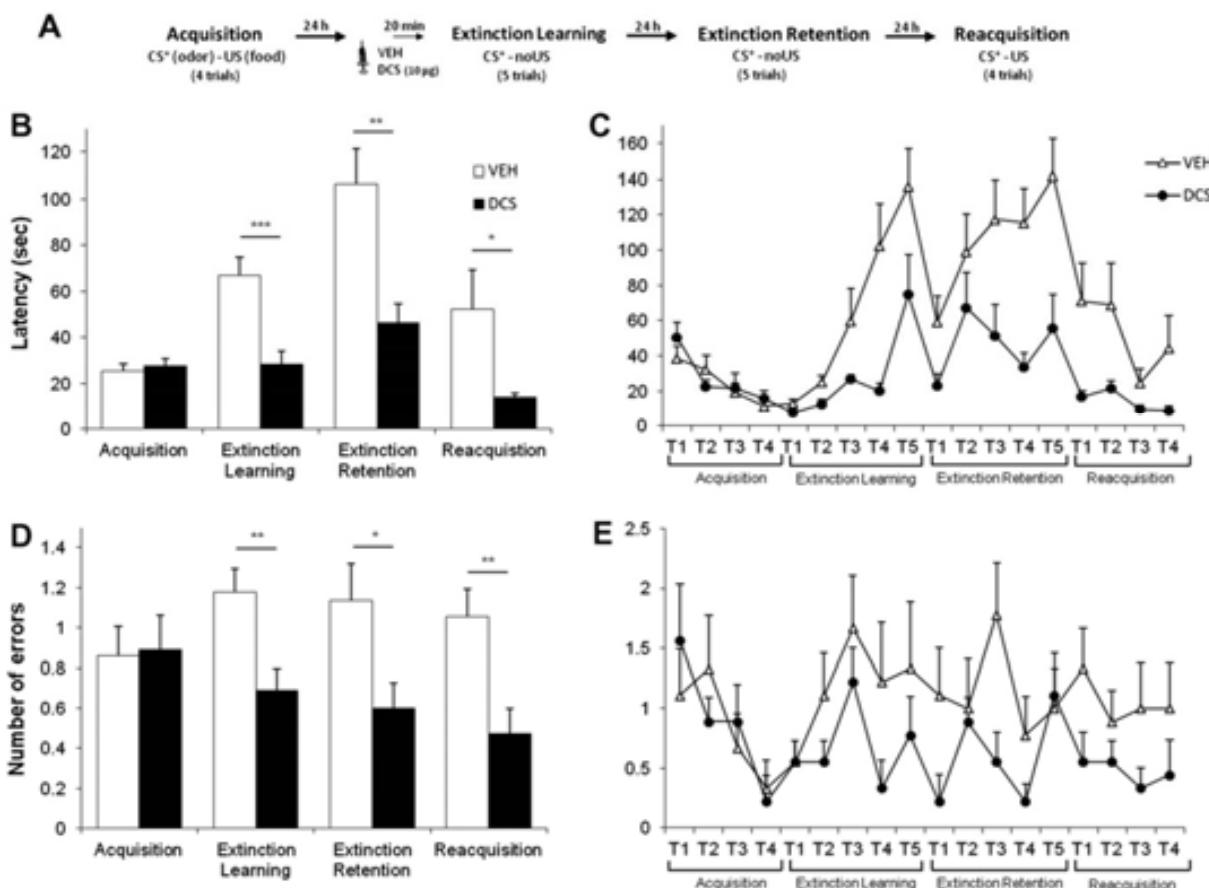
Fig. 2. Effects of pre-extinction intra-BLA DCS on the odor-reward task (experiment 1). (A) The behavioral procedure used for experiment 1. (B) Latency (average of all trials) to make the original correct response (±SEM) in each session. (C) Trial-by-trial analysis of the latency to make the original correct response. (D) Number of total errors (average of all trials) prior to making the original correct response (±SEM) in each session. (E) Trial-by-trial analysis of the number of total errors. DCS significantly decreased both measures in the extinction learning and extinction retention and improved the reacquisition of the task ($^*p < 0.05$, $^{**}p < 0.05$, $^{***}p < 0.001$), suggesting a weakened extinction learning.
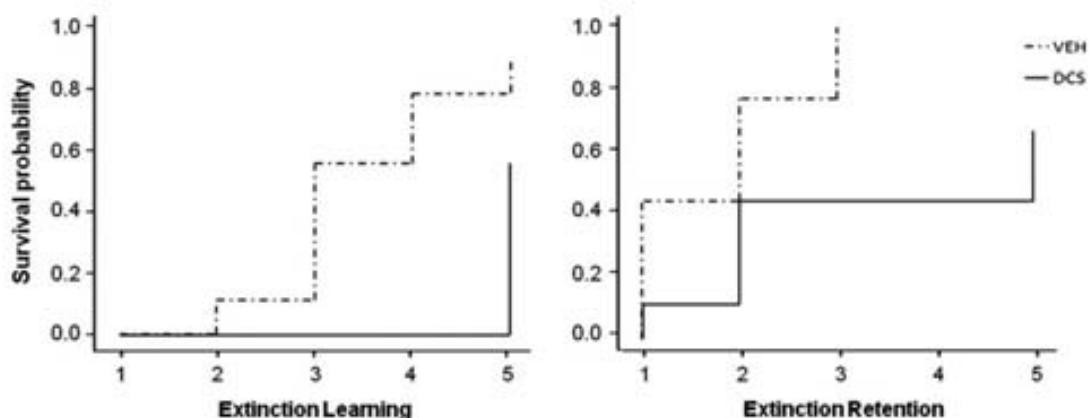
Fig. 3. Survival analysis of extinction learning and retention (experiment 1). The percentage of subjects reaching the extinction criterion (latency to make the original correct response ⩾ total mean latency of the first acquisition trial, 44 s, for two consecutive trials) is shown in the Y-axis (1 indicates that 100% of subjects acquired the criterion) for each of the 5 trials (X-axis) in each session. A significantly higher percentage of VEH subjects achieved the criterion during extinction.

group ($F_{(1,16)} = 15.585$, $p = 0.001$) and trial factors ($F_{(17,272)} = 1.679$, $p = 0.046$) were statistically significant but not the trial × group factor ($F_{(17,272)} = 0.884$, $p = 0.594$).

Poorer performance in ODT extinction did not seem to be related to changes in olfactory sensitivity since no statistically significant between-group differences were observed when the latency to find a buried sweet-smelling cookie (20 min after injection)

was analyzed a day after the completion of experimental manipulations (DCS: mean = 34.70, SE = 4.78; VEH: mean = 33.40, SE = 2.90; $F_{(1,18)} = 0.054$, $p = 0.819$).

3.2.2. Experiment 2: ODT reconsolidation

The four treatment groups depending on the two factors analyzed (drug and memory reactivation) were DCS-REACT ($n = 12$),
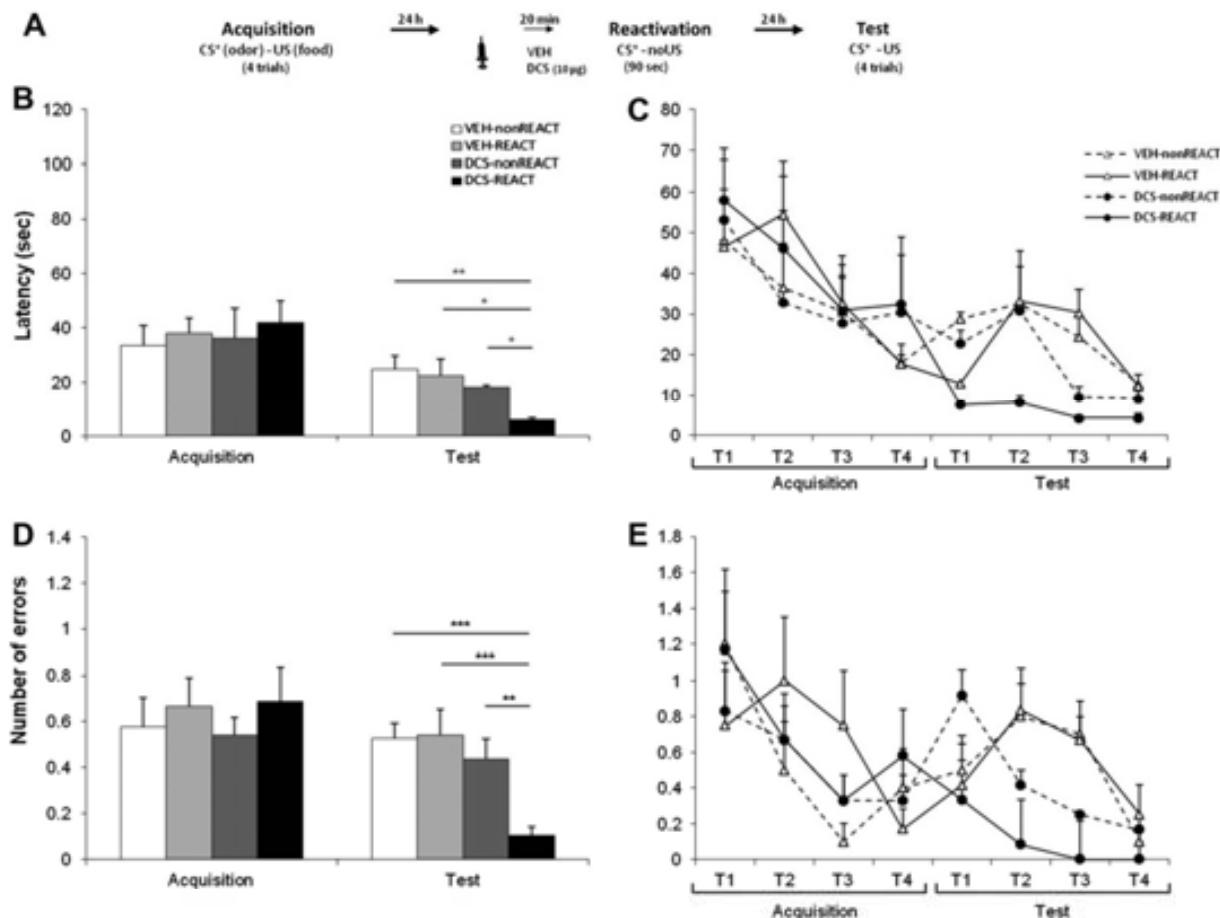
Fig. 4. Effects of pre-reactivation intra-BLA DCS on the odor-reward task (experiment 2). (A) The behavioral procedure used for experiment 2. (B) Latency to make the correct response (average of all trials) in each session (±SEM). (C) Trial-by-trial analysis of the latency to make the correct response in the acquisition and in the test sessions. (D) Number of total errors (average of all trials) prior to making the correct response (±SEM). (E) Trial-by-trial analysis of the number of total errors in the acquisition and in the test sessions. DCS in the reactivated rats significantly decreased both measures in the test session, in contrast to the remaining groups (*p < 0.05, **p < 0.05, ***p < 0.001), which indicated an enhanced reconsolidation.

VEH-REACT (n = 12), DCS-nonREACT (n = 12), VEH-nonREACT (n = 10). As depicted in Fig. 4B–E, the DCS group exposed to a memory reactivation protocol (DCS-REACT) showed shorter latencies and fewer errors than the remaining groups in the test session, suggesting an enhanced ODT reconsolidation. The analyses revealed that the session factor was statistically significant for both latencies ($F_{(1,42)}$ = 21.383, $p < 0.001$) and number of errors ($F_{(1,42)}$ = 9.510, $p < 0.01$). Since, as expected, the between-group differences were found in the test, a specific analysis for the retention session was carried out. It showed that the drug factor was statistically significant for latencies and number of errors ($F_{(1,42)}$ = 6.604, $p = 0.014$ and $F_{(1,42)}$ = 10.229, $p = 0.003$, respectively), and that the reactivation factor was not statistically significant for latencies ($F_{(1,42)}$ = 2.698, $p = 0.108$) or number of errors ($F_{(1,42)}$ = 3.788, $p = 0.06$). In addition, the interaction between drug and reactivation was statistically significant for number of errors ($F_{(1,42)}$ = 4.546, $p = 0.039$) but not for latencies ($F_{(1,42)}$ = 1.150, $p = 0.290$). Thus, regarding errors in the test session, the DCS-REACT group made significantly fewer errors than the other three groups (all $ps < 0.005$), and the DCS-nonREACT only differed from the DCS-REACT ($p = 0.005$) but not from the other groups (VEH-REACT, $p = 0.363$ and VEH-nonREACT, $p = 0.466$).

The trial-by-trial analysis of latencies (Fig. 4C) and errors (Fig. 4E) in the test session showed statistically significant effects for the group factor [($F_{(3,42)}$ = 3.508, $p = 0.023$) ($F_{(3,42)}$ = 6.338, $p = 0.001$)], and the trial factor [($F_{(3,126)}$ = 7.180, $p < 0.001$),

$F_{(3,126)}$ = 4.965, $p = 0.003$], but not for interaction [($F_{(9,126)}$ = 1.750, $p = 0.084$) ($F_{(9,126)}$ = 1.718, $p = 0.09$].

Finally, no statistically significant between-group differences were observed when the latency to find a buried cookie (24 h after injection) was analyzed two days after completing behavioral testing (DCS: mean = 45.48, SE = 2.240; VEH: mean = 43.96,SE = 2.534; $F_{(1,42)}$ = 0.202, $p = 0.655$).

4. Discussion

The results of our study indicate that the administration of DCS directly into the BLA hindered extinction and potentiated reconsolidation of odor-food reward associative memory. In the first experiment, the group receiving pre-extinction DCS showed an impaired performance, both in latencies and errors, in extinction learning and 24-h retention. Thus, DCS and VEH rats began extinction learning from a similar level of performance but diverged across subsequent trials. Furthermore, the number of trials needed to reach the extinction criterion was higher in the DCS group than in the VEH group, and in the drug-free extinction test (24 h-retention) 66.5% of DCS rats acquired the criterion in the last trial vs. 100% of VEH rats. Such resistance to extinction observed in the DCS group was also revealed in the reacquisition of the original learning after extinction training, since DCS rats displayed shorter latencies and fewer errors to make the correct response than control rats. Such

between-group differences would seem to indicate a rapid reacquisition in DCS rats, also supported by similar latencies in acquisition-reacquisition in DCS but not in VEH rats, in which latency remained longer in reacquisition than in acquisition. However, such effects should be treated cautiously as they may be magnified by the between-group differences found at the end of extinction. As there was no substantial effect of DCS on odor sensitivity, the results would indicate that, in control rats, several exposures to the reinforced odor in the absence of the reward triggered the formation of a new extinction trace encoding the dissociation between stimuli. Such new learning was attenuated in rats pretreated with a single injection of DCS into the BLA, which may be interpreted as a potentiated persistence of the original memory trace.

The findings in experiment 1 agree with other studies in animals failing to show facilitation in the extinction of appetitive tasks, specifically those reinforced with food, when systemic pre-extinction DCS injections were administered (Port & Seybold, 1998; Vurbic et al., 2011). Similarly, one study demonstrated no positive effect of systemic pre-extinction DCS on ethanol-CPP extinction acquisition, although it did report an enhanced persistence of extinction during reconditioning (Groblewski et al., 2009). Moreover, recent studies in humans have also revealed that DCS administered before extinction had no effects on the extinction of drug dependence (Price et al., 2012; Watson et al., 2011) or on a variety of behavioral disorders (Guastella, Lovibond, Dadds, Mitchell, & Richardson, 2007; Storch et al., 2010). It has also been shown that pre-extinction DCS administration facilitated fear extinction, although extinguished fear was normally renewed, suggesting that the drug may modestly facilitate extinction learning, but does not destroy the potential for relapse (Woods & Bouton, 2006).

However, the current results contrast with previous research assessing the impact of DCS administered after extinction learning, which indicated enhanced extinction consolidation of operant behavior after systemic administration in mice (Leslie et al., 2012; Shaw et al., 2009) and of different drug-seeking behaviors after intra-BLA or systemic injections (Botreau et al., 2006; Kelley et al., 2007; Nic Dhonnchadha et al., 2010; Paolone et al., 2009; Thanos et al., 2009). Thus, the moment of drug administration, i.e. before or after extinction training, would seem to be a crucial variable in the extinction of positively reinforced tasks. Moreover, additional reports have shown other inconsistencies in the capacity of DCS to enhance extinction consolidation. Such inconsistencies may be attributed to previous studies indicating that the effects of DCS may depend on the length of training (Flavell et al., 2011; Paolone et al., 2009), the anxiety levels (Tomilenko & Dubrovina, 2007), or the previous extinction level (Bouton, Vurbic, & Woods, 2008; Weber, Hart, & Richardson, 2007). The discrepancies between findings suggest that there may be fundamental methodological differences influencing the memory processes activated during retrieval of such different tasks. Therefore, the fact that DCS induces variable effects, on the grounds of its sensitivity to different procedures, supports the notion that it may not have a pervasive role as an extinction enhancer.

Further factors need to be considered in the interpretation of results regarding vulnerability to extinction, such as memory strength and age, and duration of the reactivation period (i.e. extinction training length). It has been suggested that strong memories are more resistant to extinction (Suzuki et al., 2004). In the present task, odor-reward memory may be considered as a strong memory in that it involves a survival-based behavior for finding food in deprived conditions. Be that as it may, ODT does not involve strong aversive stimuli, such as foot shocks, which may well induce stronger memories. It is also likely that older memories are less susceptible to extinction (Suzuki et al., 2004). However, in the

experiment that concerns us here, the memory was relatively recent as the acquisition session took place just one day prior to extinction learning. It has also been suggested that short CS re-exposures in the absence of US lead preferentially to reconsolidation rather than to extinction (Lee et al., 2006; Suzuki et al., 2004). Although the extinction learning in the present study may be deemed short, the protocol would appear to be sufficient as it included 5 trials lasting approximately 15 min. However, as the above three features apply to both DCS and VEH groups, with the latter showing marked signs of extinction, intra-BLA DCS may have worsened extinction learning, i.e. potentially to form a new CS-no US memory, or may have preserved the original memory.

Experiment 2 involved a brief 90-s reactivation period in which the rats were presented with the odor previously associated with a reward. The results indicated that a pre-reactivation infusion of DCS into the BLA enhanced reconsolidation in a subsequent 1-day test. The experiment also demonstrated that the mere re-exposure to the odor stimulus (VEH-REACT) or the DCS injection (DCS-nonREACT) is not sufficient to notably enhance the odor-reward memory. Nevertheless, the pattern of errors would suggest that there may have been a slight effect of DCS per se as the rats infused with the drug and not re-exposed exhibit a similar (albeit higher) function to rats infused with drug and re-exposed. However, the DCS-nonREACT group only differed from the DCS-REACT but not from the VEH groups, which may suggest that the interaction between factors, drug and brief re-exposure, is necessary to enhance reconsolidation. Additionally, DCS does not seem to have affected the reinforce intake during the reconsolidation test, as all the rats retrieved and consumed the chocolate cereal throughout the session. Thus, the results of this experiment seem to confirm the hypothesis that the administration of DCS before extinction learning in Experiment 1 may have interfered with the process of extinction by enhancing reconsolidation, since the first trial of the extinction session may have acted as a reminder. Our findings, together with other reports, identify memory reactivation as an opportunity to strengthen the memory trace by means of DCS. Thus, intra-BLA DCS has been shown to enhance the reconsolidation of stimulus-cocaine memories as it increased cue-induced relapse in rats with an extensive drug self-administration history (Lee et al., 2009). Moreover, studies carried out in single-trial paradigms have shown that DCS, injected intraperitoneally or into the BLA, potentiates fear memory reconsolidation by increasing the animals' freezing responses during the test (Lee et al., 2006; Yamada et al., 2009). Consistent with our interpretation that a positive modulation of NMDA transmission may help to stabilize the original odor-reward memory trace when reactivated, a previous study showed that intracerebroventricular blockade of NMDARs immediately after reactivation induced amnesia for the stimuli association (Torras-Garcia et al., 2005). Similarly, the administration of the NMDAR antagonist APV within the BLA prior to (but not after) a reactivation session, prevented reconsolidation of drug-associated memory, indicating that NMDARs have a temporally limited role in the reconsolidation process (Milton, Lee, Butler, Gardner, & Everitt, 2008).

Although such data suggest that DCS is able to potentiate memory consolidation after reactivation, namely reconsolidation, such an interpretation may be complicated by the fact that the injections were administered pre-reactivation (as in other reconsolidation studies, see Bustos et al., 2010; Lee et al., 2006, 2009; Yamada et al., 2009). Thus, it is possible that the effects found in the reconsolidation test may be due to performance effects on the actual reactivation trial. However, the observation of the animals' behavior during reactivation by the experimenters showed comparable olfactory bouts or motor activity between DCS- and VEH-treated animals. Such observations agree with findings from other studies showing that DCS injected before reactivation did not influence

freezing behavior during the reactivation session of fear conditioning (Bustos et al., 2010; Lee et al., 2006). Nevertheless, post-session injections would have better isolated retrieval-induced memory processes, and the possibility that post-reactivation DCS affect ODT reconsolidation should be addressed in future studies. In the interpretation of results from experiment 2, we should also consider the importance of the relation between the glutamatergic transmission in the BLA and reward-related processes. Thus, rapid transient fluctuations in glutamate in the BLA tended to precede lever-pressing actions for sucrose reward and were markedly increased in frequency when rats were engaged in such reward-seeking actions (Wassum et al., 2012). Moreover, GluR1-containing glutamate receptors are important in the synaptic plasticity in the BLA that underlies conditioned reinforcement (Mead & Stephens, 2003). Accordingly, the present findings may support the notion that DCS, by increasing glutamate transmission in the BLA, can potentiate synaptic plasticity strengthening the rewarding properties of the odor during the reactivation session, thus resulting in enhancement of the conditioned response, i.e., reducing latency and number of errors to find the target CS.

Although DCS is a commonly studied cognitive enhancer, its mechanisms of action are not completely understood (Davis et al., 2006). Nevertheless, as plasticity in the BLA has been shown as an important factor for reconsolidation and extinction of emotional memories (Duvarci et al., 2006), the effects of DCS have been widely interpreted in terms of modulation of neuroplasticity (Myers & Davis, 2002). Thus, DCS-induced NMDAR efficacy enhancement, by stimulating high affinity glycine binding, may possibly modify NMDAR-mediated intracellular events (Norberg, Krystal, & Tolin, 2008). NMDA agonists and other cognitive enhancers may trigger a signaling cascade resulting in AMPA receptor subunit internalization in the amygdala and enduring alterations in synaptic transmission resulting in changes in the number and morphology of dendritic spines at cortical inputs to amygdalar neurons (Mao, Lin, & Gean, 2008; Yang, Chao, Ro, Wo, & Lu, 2007). Particularly noteworthy in such a context is the report that activation of amygdalar protein kinase A, a key component of the synaptic plasticity machinery, enhanced fear memory when the memory was briefly reactivated but not extinguished (Tronson, Wiseman, Olausson, & Taylor, 2006).

A final issue to be considered is that, although the BLA has been extensively related to extinction and reconsolidation of different tasks, other brain regions may also participate in such processes. In this context, several studies have implicated the hippocampus in reconsolidation (Nader & Hardt, 2009; Tronson & Taylor, 2007) and the medial prefrontal cortex, which modulates amygdalar activity, in reconsolidation and extinction (Akirav & Maroun, 2006; Milton & Everitt, 2010; Myers & Davis, 2007; Quirk & Mueller, 2008). As for ODT, a study using c-fos immunocytochemical marking showed that a circuit linking BLA and the prefrontal cortex is involved in the post-acquisition consolidation period of the odor-reward association (Tronel & Sara, 2002). Also, NMDARs in PLC are necessary in the early stage of ODT consolidation as blockade of such receptors immediately after training induced long-lasting amnesia (Tronel & Sara, 2003). This agrees with the demonstration that bilateral DCS administration in the PLC enhanced subsequent ODT memory expression in a relearning test (Villarejo-Rodriguez et al., 2010). The involvement of this region in ODT has also been suggested in a recent study showing that intra-PLC DCS attenuated learning deficits induced by thalamic lesions (Villarejo-Rodriguez et al., 2012). Considering this evidence, it is likely that DCS infused in regions anatomically related to BLA and also involved in this task, such as the prefrontal cortex, affect ODT extinction and/or reconsolidation. Hence, additional studies would need to investigate the effects of DCS infusions into different brain regions in order to further explore the neural mechanisms involved in ODT extinction and reconsolidation. Additionally, other circumstances would need to be examined to elucidate the potential use of DCS, such as re-exposure protocol or dose and moment of injection. For instance, control infusions of DCS several hours after retrieval would be useful to assess the effects of the drug *per se* on subsequent retention tests.

5. Conclusions

The results presented corroborate the involvement of the NMDARs in an appetitive odor-food reward task (Tronel & Sara, 2003), and particularly its strychnine-insensitive glycine-binding site (Villarejo-Rodriguez et al., 2010). Specifically, the results show that intra-BLA DCS may exert opposite effects in ODT extinction and reconsolidation, namely, impairment of extinction and enhancement of reconsolidation. Such results suggest that DCS into the BLA may potentiate the persistence or strength of the original odor-reward memory trace (demonstrated by resistance to extinction and facilitation of reacquisition and reconsolidation), which is compatible with the idea that memory retrieval is a dynamic process that either reinforces or alters memory (Suzuki et al., 2004). Therefore, studies of DCS or other agents that may act specifically on extinction or reconsolidation are particularly interesting in order to avoid problematic potential side effects in the therapies used to treat neurologic or psychiatric disorders.

Acknowledgments

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2.   Experiments 3 and 4: Efectes de la DCS al CPL per revertir dèficits produïts per l'SCOP en la DSO i la TSPA

D-cycloserine in prelimbic cortex reverses scopolamine-induced deficits in olfactory memory in rats

Portero-Tresserra M, Cristóbal-Narváez P, Martí-Nicolovius M, Guillazo-Blanch G, Vale-Martínez A.

2.1   Theoretical approach

It has clearly been shown that glutamatergic and cholinergic systems are involved in learning and memory processes. Pharmacological experiments have pointed particularly muscarinic receptors to have a critical role in the involvement of the cholinergic system in memory (Bartus et al. 1982) and its blockade has been considered a pharmacological model for cognitive impairment (Klinkenberg & Blockland 2010). Thus, administration of muscarinic antagonists, such as scopolamine (SCOP), produces a deficit in several learning tasks, such as ODT and social transmission food preference (STFP) (Carballo-Marquez et al. 2009). In addition, extensive research has also been conducted to investigate glutamatergic NMDAR mechanisms underlying to learning and memory processes (Castellano et al. 2001), since NMDAR functioning is essential for synaptic plasticity and memory (Malenka et al 1998). Studies with NMDAR antagonists have found learning impairments and the administration of DCS has demonstrated cognition-enhancing properties. Moreover, there are some lines of evidence suggesting the interaction between cholinergic and glutamatergic systems in modulating memory processes. For instance, pharmacological studies indicated that the concomitant administration of subeffective doses of SCOP and MK-801, a NMDAR antagonist, produces a deficit in spatial and emotional learning (Li et al. 1997; Hlinak and Krejci 1998). Furthermore, systemic and intra-hippocampal infusions of DCS attenuated SCOP-induced deficits in several learning tasks. Nevertheless, a better understanding of the critical brain structures in which the interaction may occur is needed.

The present study is supported by the hypothesis of an interaction between cholinergic and glutamatergic pathways in the prelimbic cortex (PLC) in the modulation of olfactory memory. For this reason, in this study two experiments were conducted, aimed at evaluating the effects of DCS and SCOP in the PLC, on two different olfactory learning paradigms, ODT and STFP. As in the retention test performed in the STFP task (experiment 3), DCS did not facilitate memory in SCOP-untreated rats and the reversion of SCOP-induced deficits was less noticeable than in the ODT, another experiment was included in which the number of choice alternatives was augmented to three in the STFP test. This 3-choice memory test may elude a potential ceiling effect in the percentage of food preference and also it may increase the task difficulty which enhances the participation of the frontal cortex (Winocur et al. 2001). This brain region, which includes PLC, is particularly related to cognitive flexibility and behavioral inhibition in the decision-making process (van Kerkhof et al. 2013). Therefore, the potentiation of NMDA transmission would be more efficient to enhance memory under more demanding conditions.

- Experiment 3: The role of the DCS injection in the PLC was analyzed in compensating dysfunction of PLC muscarinic receptors due to SCOP administration. Thus, DCS was administrated 20 minutes prior to ODT and STFP acquisition, SCOP was injected immediately afterwards, and memory was tested in subsequent 24-hour retention tests.

- Experiment 4: Similarly to the experiment 3, the effects of DCS administration into the PLC to reverse SCOP-induced memory deficits were evaluated. Nonetheless, in this study the number of choice options in the STFP 24-hour retention test was increased to three.

2.2 Experiment 3

2.2.1 Methods and procedure (Figure 23)

The objective of this experiment was to explore whether DCS infusions into the PLC may compensate the deficits produced by PLC muscarinic blockade in ODT and STFP memory. Hence, 20 minutes before the ODT and STFP acquisition, the rats received bilateral intra-PLC infusions of DCS (10µg/side) or VEH. Immediately afterwards, intra-PLC SCOP (20µg/side) or VEH were administrated. The DCS dose were based on previous research in which intra-PLC DCS enhanced ODT (Villarejo-Rodriguez et al. 2010) and SCOP disrupted ODT and STFP memory (Carballo-Marquez et al. 2009). Twenty four hours later, a drug-free retention test was performed in both tasks.

To carry out this experiment, the final sample was made up of 39 Wistar rats which were distributed into four experimental groups. For the ODT, VEH=9, DCS=10, SCOP=9, DCS+SCOP=11 and for the STFP, VEH=10, DCS=10, SCOP=8, DCS+SCOP=11. All animals underwent ODT and STFP in a counterbalanced way (half sample: ODT-STFP, half sample: STFP-ODT). At the beginning of the experiment, the subjects were habituated to the reinforcement (crispy chocolate cereal) and to the training box for ODT, and they were familiarized to powdered chow in glass jars for STFP. Afterwards, they underwent sterotaxic implantation of bilateral chronic guide cannulae in the PLC. Once the rats were recovered for the surgery, they were submitted to another habituation session and they were also adapted to a mock infusion protocol. After one week, the ODT acquisition (4 trials) was conducted according to procedures explained in experiments 1 and 2. Twenty minutes before ODT acquisition and immediately after, the rats were injected with DCS/VEH or SCOP/VEH and for this procedure they were immobilized and microinjectors were introduced through the cannulae guide (Plastics One). Thus, first the subjects received PBS (phosphate-buffered saline 0.1 M PH 7.4) or 10µg DCS (Sigma-Aldrich) dissolved in PBS and after they received PBS (phosphate-buffered saline 0.1 M PH 7.4) or 20µg SCOP (Sigma-Aldrich) dissolved in PBS. The solutions were infused bilaterally in a volume of 0.5 µl/hemisphere for 2 minutes and a rate of 0.25µl/min. The STFP acquisition started when a demonstrator was allowed to eat flavored food (with 2.2% cocoa or 1% cinnamon), and following a period of 30 minutes, the demonstrator that had just eaten was placed into the observer's cage and the two rats were allowed to interact for 30 minutes. One day after the STFP acquisition, all observers were tested during 45 minutes in a retention test by placing two jars filled with odorized food, one with the flavor that the demonstrator had eaten (trained food) and the other contained new chow (untrained food). A preference score (percentage eaten of trained food) for the trained food was calculated as a measure of learning. During the STFP acquisition and testing,

other variables were also scored such as social interaction measures, motivation to eat, exploration and neophobic reactions. Finally, as it was described in the experiments 1 and 2, an additional olfactory perception test was conducted. Twenty minutes before the habituation to the flavored cookie the rats were infused with DCS or PBS and immediately after with SCOP or PBS. Twenty four hours later, they were placed in a cage, where a piece of cookie was buried, and the latency to find it was recorded.
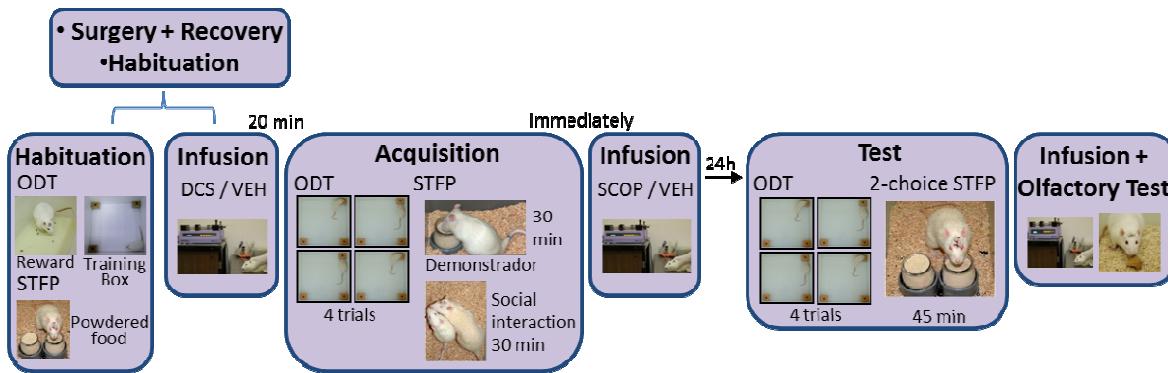
Figure 23. The behavioral procedure used in the experiment 3

2.2.2 Summary of the main results

- The NMDAR co-agonist DCS administered into the PLC before acquisition rescued ODT memory impairment induced by SCOP. The group DCS+SCOP showed shorter latencies and less number of errors during the 24-hour retention test than the SCOP group and their performance did not significantly differ from that of the VEH group.

- The injection of DCS into the PLC prior to the acquisition ameliorated SCOP-induced STFP memory deficits in a standard 2-choice STFP test, since the DCS+SCOP group showed a preference score superior to the chance level although it did not reach the same level than the VEH group.

- The intra-PLC infusion of DCS alone before acquisition enhanced ODT memory retention as the group treated with DCS performed shorter latencies and fewer errors on the 24 hours retention test than the VEH rats. However, DCS did not produce any effect in the 2-choice STFP retention test, since DCS and VEH groups exhibited an equivalent performance.

- Intra-PLC administration of SCOP after the ODT / STFP acquisition disrupted memory in both retention tests. SCOP groups displayed larger latencies and more number of errors during the ODT retention test and they showed a food preference around 50% on the STFP retention test.

- The infusions of DCS and SCOP did not produce any effect on olfactory perception, social investigation, neophobic responses or motor activity

2.3   Experiment 4

2.3.1   Methods and procedure (Figure 24)

The results from the experiment 3 indicated that DCS did not facilitate memory in SCOP-untreated rats in the standard 2-choice paradigm. Moreover the reversion of the SCOP induced deficits in the STFP was less marked than in the ODT. Thus, in experiment 4 the STFP 24 hours-retention test was modified by presenting the trained food along with two equally palatable alternatives. A memory test containing three response options allows a wider scope for observing performance improvement due to DCS infusion. Likewise, the use of more choices may increase the task difficulty and, in consequence, the participation of the prefrontal cortex. Specifically, earlier studies confirmed that rats with lesions in the frontal cortex displayed anterograde amnesia only when the STFP protocol included three options in the memory test (Winocur & Moscovith 2009).

On this experiment, a final sample of 44 male Wistar rats was used as observers and they were included in four different experimental groups: VEH=11, DCS=11, SCOP=11, DCS+SCOP=11. During this experiment all the procedures where the same as those in the experiment 3, with the exception that in the habituation and the test session the food tray contained three jars and an additional flavored food was included (0.5% vanilla). The variables registered were the same as in the experiment 3 and a similar olfactory test was used.
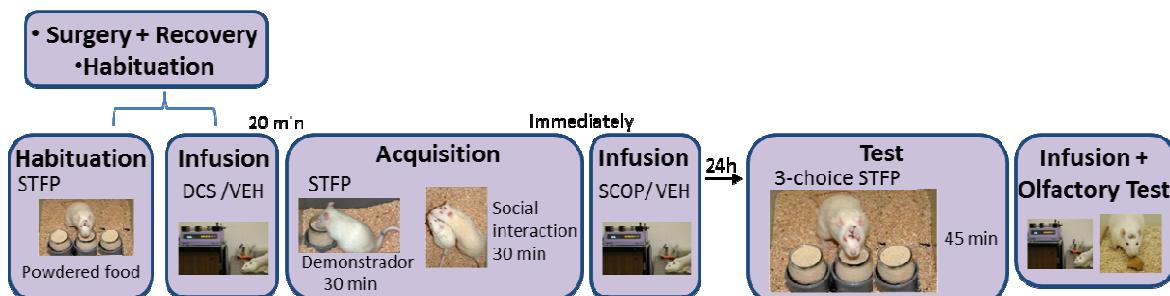
Figure 24. The behavioral procedure used in the experiment 4

2.3.2 Summary of the main results

- The NMDAR co-agonist DCS administered into the PLC prior to the acquisition rescued memory impairments in the 3-choice STFP test induced by SCOP. The group DCS+SCOP showed a higher preference for the trained food than the SCOP group, and their performance was not different from that of the VEH group.

- The administration of DCS alone into the PLC did not produce enhancing effects on the 3-choice STFP retention test, similarly to what was found in experiment 3.

- The administration of SCOP into the PLC after the STFP acquisition induced marked memory impairment on the 3-choice retention test and the SCOP-treated rats scored a percentage of food preference around chance level.

- The DCS and SCOP administration, as described in previous experiments, did not affect the ancillary variables registered during the STFP acquisition and testing or the olfactory test.

Experimental Research

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D-cycloserine in Prelimbic Cortex Reverses Scopolamine-Induced Deficits in Olfactory Memory in Rats

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Abstract

A significant interaction between N-methyl-D-aspartate (NMDA) and muscarinic receptors has been suggested in the modulation of learning and memory processes. The present study further investigates this issue and explores whether d-cycloserine (DCS), a partial agonist at the glycine binding site of the NMDA receptors that has been regarded as a cognitive enhancer, would reverse scopolamine (SCOP)-induced amnesia in two olfactory learning tasks when administered into the prelimbic cortex (PLC). Thus, in experiment 1, DCS (10 µg/site) was infused prior to acquisition of odor discrimination (ODT) and social transmission of food preference (STFP), which have been previously characterized as paradigms sensitive to PLC muscarinic blockade. Immediately after learning such tasks, SCOP was injected (20 µg/site) and the effects of both drugs (alone and combined) were tested in 24-h retention tests. To assess whether DCS effects may depend on the difficulty of the task, in the STFP the rats expressed their food preference either in a standard two-choice test (experiment 1) or a more challenging three-choice test (experiment 2). The results showed that bilateral intra-PLC infusions of SCOP markedly disrupted the ODT and STFP memory tests. Additionally, infusions of DCS alone into the PLC enhanced ODT but not STFP retention. However, the DCS treatment reversed SCOP-induced memory deficits in both tasks, and this effect seemed more apparent in ODT and 3-choice STFP. Such results support the interaction between the glutamatergic and the cholinergic systems in the PLC in such a way that positive modulation of the NMDA receptor/channel, through activation of the glycine binding site, may compensate dysfunction of muscarinic neurotransmission involved in stimulus-reward and relational learning tasks.

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Introduction

It has been extensively demonstrated that the cholinergic and glutamatergic systems are involved in cognitive processes, and some lines of evidence suggest an interaction between muscarinic and N-methyl-d-aspartate receptors (NMDARs) in the regulation of learning and memory [1], which has mainly been assessed in the hippocampus. Thus, in vitro studies showed that the activation of muscarinic receptors increased the probability of generating NMDA-dependent long-term potentiation (LTP) [2]. Moreover, the acute application of memantine, a non-competitive NMDA receptor (NMDAR) antagonist approved for the treatment of Alzheimer's disease, caused a significantly enhanced synaptic transmission in hippocampal slices that was blocked by the muscarinic antagonist scopolamine (SCOP) [3].

Behavioral pharmacological studies also suggest that these systems may work interactively. Firstly, systemic concomitant administration of ineffective doses of muscarinic and NMDAR antagonists produced amnesic effects in several tasks, such as spatial mazes [4,5], contextual fear conditioning [1], inhibitory avoidance [6,7] and a visual recognition memory task [8]. Recent studies confirmed significant deficits when sub-threshold doses were co-administered intracerebrally. Specifically, injections in the

medial septum or CA1 [9] or the ventral tegmental area [10] induced amnesia in inhibitory avoidance. Secondly, systemic pre-learning infusions of d-cycloserine (DCS), a NMDAR partial agonist at the glycine modulatory site that enhances memory processes [11,12,13,14,15], attenuated SCOP-induced deficits in the acquisition of spatial tasks [16,17,18,19]. Additionally, it has been shown that acute application of memantine also reversed SCOP-induced learning impairments in the water maze [3]. In non-spatial paradigms, DCS injected prior to retention reduced the negative effects of SCOP on brightness discrimination [20] and visual recognition [21]. As for intracerebral studies, early research suggested that the hippocampus may be involved in the muscarinic/NMDA interaction as reversal, with DCS, of SCOP-induced deficits in spatial working memory was found when both drugs were injected into the hippocampus [22,23]. Furthermore, a recent report showed that injections of NMDA into the medial septum, a main hippocampal afferent, reduced SCOP-induced amnesia in inhibitory avoidance [24].

Nevertheless, a better understanding is needed of the critical brain structures in which the proposed systems interaction may occur in modulating memory. Therefore, in the present study we evaluated the effects of SCOP and DCS injected into the prelimbic cortex (PLC) as the previous literature suggests that this cortical

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region may be a suitable candidate. In this regard, a homogenous distribution of both glutamatergic and acetylcholinergic innervation has been described in the PLC [25]. It has also been shown that muscarinic agonists modulated the amplitude of the excitatory postsynaptic potentials, mediated by glutamate receptors, in 100% of the PLC neurons tested [26]. Previous reports also demonstrated that intra-PLC administration of SCOP disrupted memory assessed in associative paradigms based on olfaction, such as social transmission of food preference (STFP) [27,28] and odor discrimination (ODT) [29] tasks. This is especially relevant as smell loss and pathological involvement of the olfactory pathways are present in the formative stages of neurodegenerative diseases [30]. Moreover, the ODT paradigm is sensitive to the beneficial effects of intra-PLC DCS, as an acute pre-learning treatment improved the performance of non-lesioned rats [31] and rats with thalamic lesions [32]. Both learning tasks are naturalistic appetitive forms of associative memory and independent of spatial information [33], but differ in some of the structures and underlying memory systems on which they rely. ODT mostly depends on a network of closely related brain regions, particularly in the prefrontal cortex (PLC, infralimbic, orbital) and the amygdala [34,35] and STFP is related to the prefrontal cortex [28,36] and amygdala [37] but also to the hippocampal formation [33,38,39,40,41].

The purpose of the present study was to examine whether NMDAR activation in the PLC may compensate dysfunction of PLC muscarinic neurotransmission assessed in two olfactory learning tasks with a differential involvement of the hippocampus. As in previous studies [28,29,31,32], an acute DCS treatment was administered 20 min before ODT and STFP learning, SCOP was injected immediately afterwards, and memory was assessed in a subsequent 24-h retention test (experiment 1). As in the standard 2-choice STFP paradigm (experiment 1) DCS did not facilitate memory in SCOP-untreated rats and the reversion of SCOP-induced deficits was less conspicuous than in the ODT, a second experiment was performed in which the number of choice alternatives in the STFP test was increased to three. A test containing more response options may elude a potential ceiling effect in the percentage of food preference as it allows a wider scope for observing performance improvements due to DCS administration. Moreover, the inclusion of more distracter foods may increase the task difficulty and the engagement of the frontal cortex [42,43]. This structure, including the PLC, is particularly related to cognitive flexibility and behavioral inhibition in the decision-making process (during the selection response) [44,45], which may suggest that potentiating NMDA transmission would more effectively enhance memory in demanding conditions.

Materials and Methods

Ethics Statement

All procedures were carried out in compliance with the European Community Council Directive for care and use of laboratory animals (86/609/European Community Council) and with the Generalitat de Catalunya's authorization (Diari Oficial de la Generalitat de Catalunya 2450 7/8/1997, Departament d'Agricultura Ramaderia i Pesca protocol number 5959).

Experiment 1: ODT and Two-choice STFP

Subjects. Forty-six Wistar male rats belonging to our laboratory's breeding stock were used (mean age = 97.7d, SD = 4.28; mean weight = 378.95g, SD = 26.72 at the beginning of the experiment). An additional set of 41 male Wistar rats (mean age = 57.45 d, SD = 4.68; mean weight = 259.54 g, SD = 42.45 at

the beginning of the experiment) served as demonstrator subjects in the STFP task. All the rats were single-housed in 50×22×14 cm plastic-bottomed, sawdust-bedded cages in a room controlled for temperature (20–22°C) and humidity (40%–70%). The rats were maintained on a 12 h light-dark cycle (lights on at 8:00 a.m.), with experiments performed during the light phase of the cycle. Ratchow pellets (Scientific Animal Food & Engineering, Augy, France) and water were provided *ad libitum* except during habituation, acquisition and test sessions, in which the rats were submitted to a food restriction schedule (12 g/d to maintain body weight at 85% of their free-feeding weight). The animals were handled on a daily basis for 5 min and restrained for 2 min to accustom them to the injection procedure.

Surgery. Animals were anesthetized and underwent stereotaxic implantation of bilateral chronic double-guide cannulae into the PLC following procedures explained in detail elsewhere [28], and all efforts were made to minimize suffering. Each guide cannula comprised two 26-gauge metal tubes projecting 2.9 mm from the pedestal (Plastics One, Bilaney Consultants GMBH, Düsseldorf, Germany.). The stereotaxic coordinates for implantation in the PLC were (Fig. 1A): AP, +3.5 mm from bregma; ML, ±0.6 mm from midline; and DV, −2.9 mm from cranium surface [46]. Sterile dummy stylets (Plastics One) were placed into the cannulae to prevent occlusion. After surgery, rats were administered an antibiotic (Panolog, Novartis) and were returned to their home cages for 10 days (4 for recovery, 4 for food restriction and 2 for rehabituation) before behavioral training. During the 10-day recovery period, the rats were handled and weighed on a daily basis and the dummy stylets were changed every other day.

Microinfusion procedure. The rats received the drug infusions twenty min before (DCS/vehicle) and immediately after ODT and STFP acquisition (SCOP/vehicle) (Figs. 2A and 3A). For this purpose, they were gently restrained while the dummy stylets were removed and replaced with 33-gauge stainless-steel double injectors (Plastics One) extending 1 mm below the cannula tips. The injectors were connected by polyethylene tubing (Plastics One) to two 10-μl syringes (SGE Analytical Science, Cromlab S.L. Barcelona, Spain) mounted in an infusion pump (11 Plus Syringe Pump, Harvard Apparatus Inc., Holliston, Massachusetts, USA). DCS (Sigma-Aldrich, Madrid, Spain) and SCOP (Scopolamine Hydrobromide USP, Sigma–Aldrich Quimica S.A., Madrid, Spain) were dissolved in PBS (phosphate-buffered saline 0.1 M, pH 7.4) and doses of 10 μg/hemisphere (DCS) and 20 μg/hemisphere (SCOP) were administered into the PLC. The rats in the control VEH groups received vehicle (PBS) injections. The solutions were infused bilaterally in a volume of 0.5 μl/hemisphere for 2 min. The injectors were left in place for 1 min after the infusion was complete to allow for diffusion. The dose, volume and injection time of the drugs were based on previous studies in which intra-PLC DCS enhanced ODT [31,32] and SCOP disrupted ODT and STFP memory [28,29].

Apparatus. In the ODT, the habituation to reinforcement was performed in a plastic bottomed cage (50×22×14-cm). The training apparatus was a 60×60×40 cm square box containing three sponges with a 3-cm diameter hole cut into the centre, placed in glass slide-holders of the same size [47]. The food reinforcement used was a crispy chocolate rice breakfast cereal (Kellogg's, Spain) that was placed at the bottom of the opening in the sponge. Each sponge was infused with an odor that was injected into all its corners. The odors, vanilla (0.3 ml), orange (0.6 ml) and anise (0.2 ml) (Vahiné, Ducros S.A., Sabadell, Spain), were previously tested in a pilot study in which the rats showed no particular preference. All behavioral sessions were recorded by a
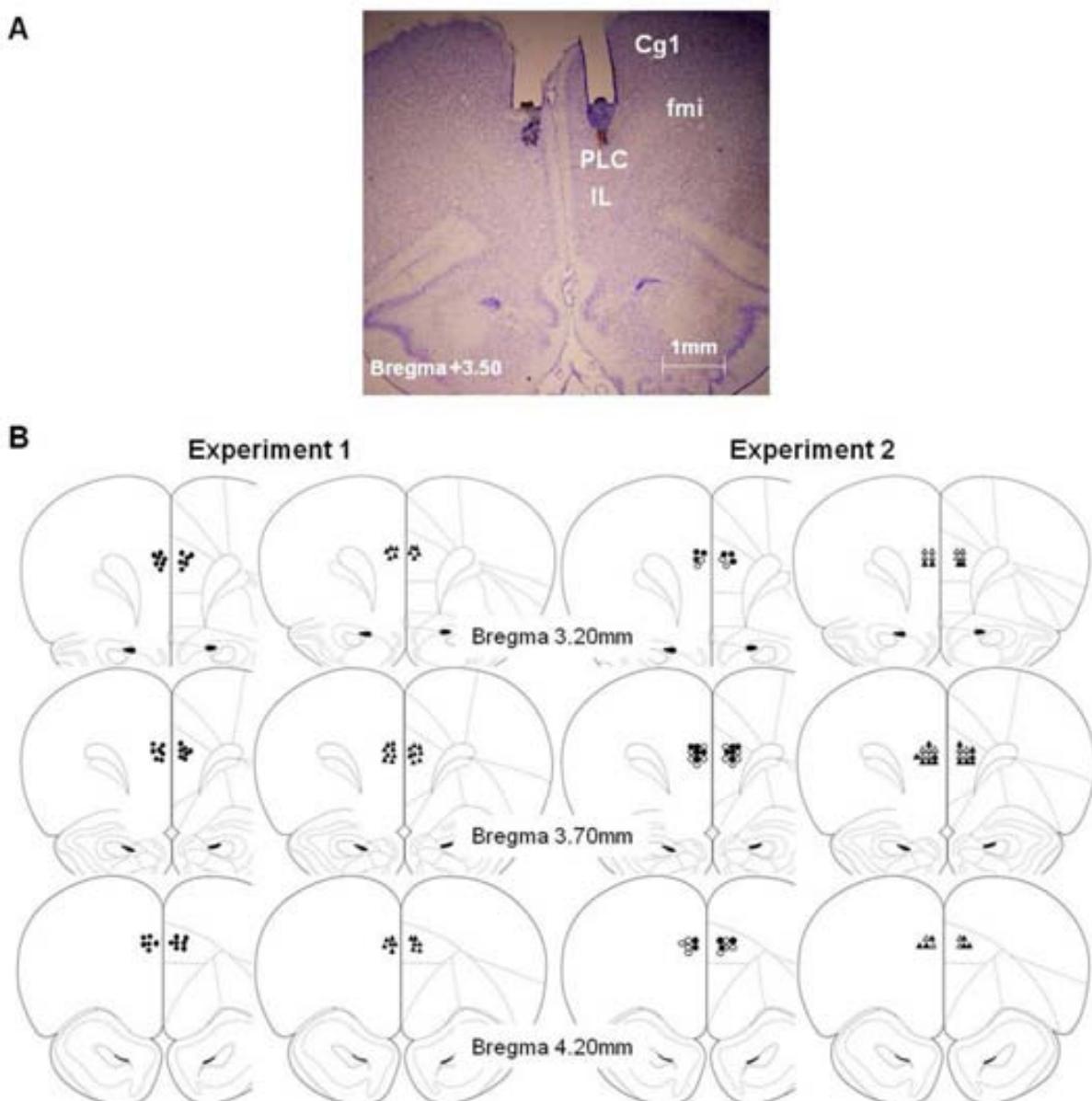
Figure 1. Histology. A) Photomicrographs of Cresyl violet staining at the level of the PLC area (AP, 3.50 mm anterior to bregma) showing the cannula track and the micro-injector tip of a representative subject [Cg1, cingulate cortex area 1; Fmi, forceps minor of the corpus callosum; IL, infralimbic cortex; PLC, prelimbic cortex] (B) Micro-injector tip placements throughout the rostral-caudal extent of the PLC (Paxinos and Watson, 1997) in experiment 1 (DCS and DCS+SCOP are represented by filled circles; VEH and SCOP by filled triangles) and experiment 2 (VEH is represented by empty circles; DCS by filled circles; SCOP by empty triangles; DCS+SCOP by filled triangles).
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video camera (JVC, Everio Model GZ-X900) connected to a monitor.

In the STFP task, all observers were habituated, trained and tested in their own 50×22×14-cm plastic-bottomed, sawdust-bedded cages. Habituation and testing were carried out using a feeding-tray placed in the animals' cages. The tray consisted of a black Plexiglas base (21×21-cm) with two adjacent plastic pots fixed onto the center of the base. The food (powdered rat chow) was placed in glass jars (130 ml) secured within each plastic pot. For the demonstrators, habituation and acquisition were carried out in 50×22×14-cm plastic cages in which they were allowed to eat from a glass jar mounted upon the center of a black Plexiglas base (21×10-cm). For the acquisition and test, powdered rat chow was 2.2% ground cocoa (Oxfam Fairtrade, Gent, Belgium) and/or 1% ground cinnamon (Carmencita, Alicante, Spain). All sessions were recorded by a video camera (JVC, Everio Model GZ-X900) connected to a monitor.

Behavioral procedure: ODT. All the animals underwent ODT and STFP in a counterbalanced way (half sample: ODT-STFP, half sample: STFP-ODT). The injections were also counterbalanced, with the subjects administered with DCS before the first task acquisition receiving VEH in the second one, and those administered with SCOP after the first task acquisition receiving VEH in the second one.
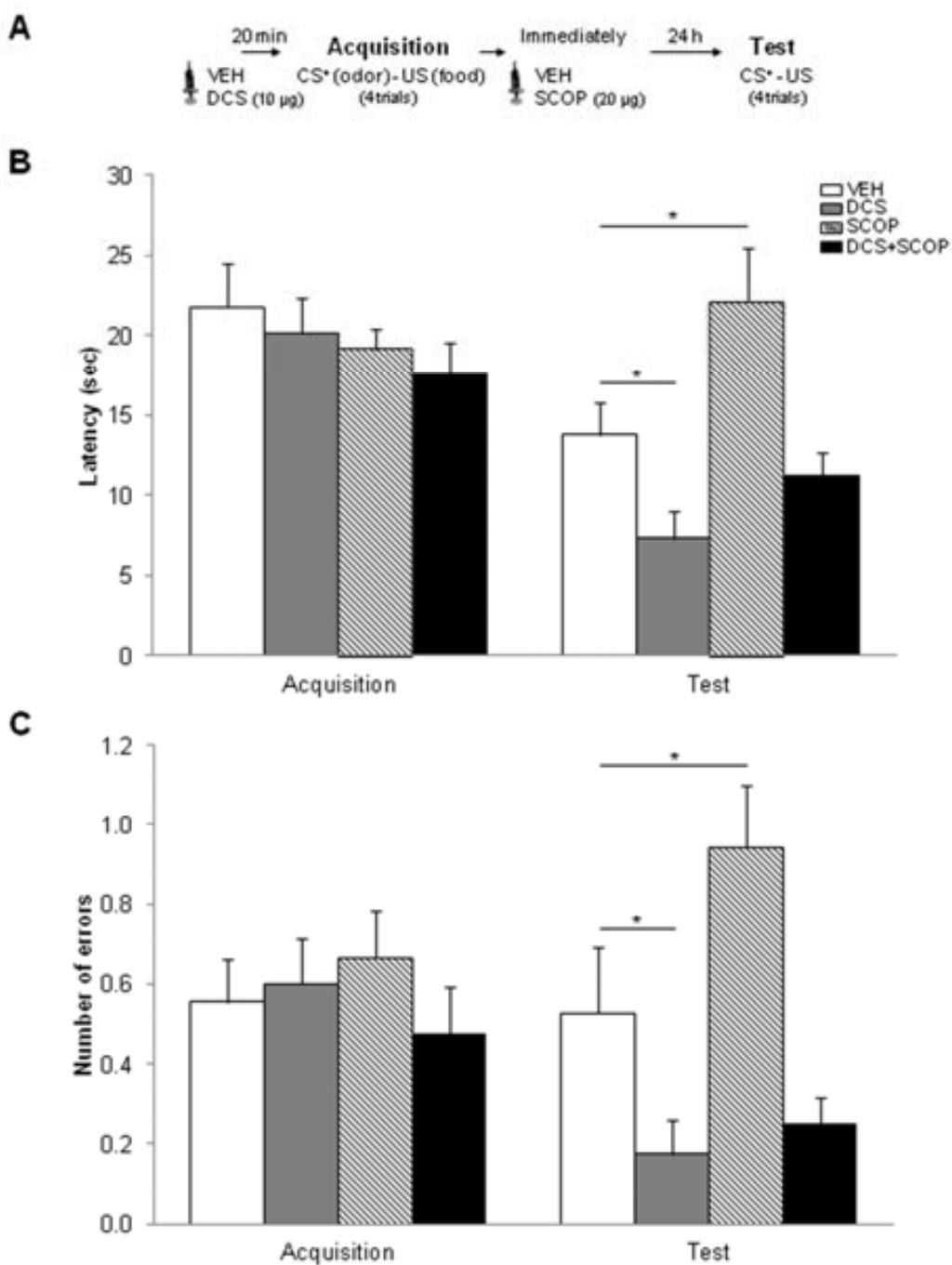
Figure 2. Experiment 1 (ODT). (A) The behavioral procedure used for experiment 1. (B) Latency (average of all trials) to make the correct response (±SEM) in each session. (C) Number of total errors (average of all trials) prior to making the correct response (±SEM) in each session (*p<0.05).
doi:10.1371/journal.pone.0070584.g002

The rats were food-restricted for five days prior to three pre-surgery habituation sessions in which they were familiarized with the reinforcement and the training box. After consuming ten pieces of cereal/session, they were placed in the training box, without the reinforcement, and allowed to explore it for 15 min. Six days after surgery, rats were again food-restricted and submitted to an identical 15-min rehabituation session and a mock infusion protocol (no solutions injected) in order to minimize any stress associated with the procedure.

One day after rehabituation, ODT acquisition was carried out in a single four-trial session (Fig. 2A), according to procedures previously described [31]. The reinforcement (chocolate rice cereal placed at the bottom of the opening in the target sponge) was associated with the same odor across trials, and the target odor was randomly assigned to each rat in a counterbalanced way. The sponges with the non-reinforced odors did not contain any food. Sponges were placed in any three of the four corners of the box, and the position of each odor within the box was changed for each trial according to a previously determined protocol.
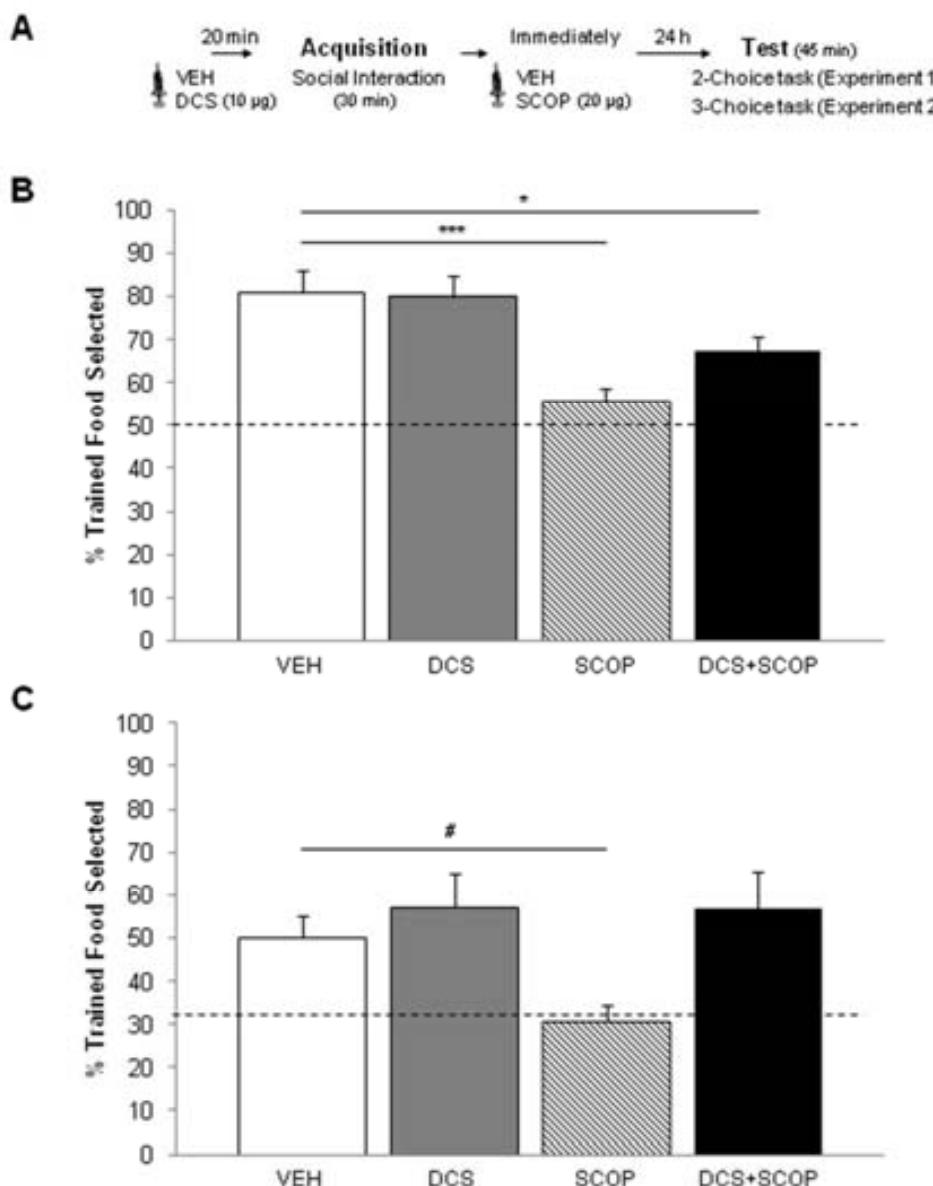
Figure 3. Experiments 1 and 2 (STFP). (A) The behavioral procedure used for experiment 1 and experiment 2. (B) Percentage of trained food selected, expressed as the mean percentage ($\pm$ SEM) of the total amount of food consumed in the STFP two-choice test (experiment 1) and (C) the three-choice test (experiment 2) (*$p < 0.05$,***$p < 0.0001$, #$p = 0.06$).
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The rats were placed in the training box, facing the corner with no sponge. There was a 3-min limit for the rats to find and consume the reinforcement and the inter-trial interval was 1 min. Latency before a correct response (nose-poking into the target sponge) and errors were scored. Two different errors were combined: errors of commission (nose-poking into a non-target sponge) and omissions (sniffing the target sponge not followed by nose-poking) [35]. Latencies and errors were scored by two independent judges that were blind to drugs administration.

Twenty-four hours after acquisition, the rats were tested (24h-test session) using the same procedure as in the previous acquisition session. The first test trial was not reinforced to measure memory of the previous training [35].

Behavioral procedure: Two-choice STFP. After 5 days of food restriction, prior to surgery, observers and demonstrators were habituated to powdered chow (Scientific Animal Food & Engineering, Augy, France) from glass jars to minimize neophobia, for 2 h on the first day, 1 h the second day and 45 min the third day. The rats were presented with food cups in feeding trays containing ground, unflavored rat-chow, in their own cages. A similar procedure was repeated 6 days after surgery for the observers (two 45-min rehabituation sessions). Subsequently, animals were food-restricted once again for 2 days before the training–testing sessions began.

The STFP acquisition and test were conducted following procedures explained elsewhere [27,28]. Essentially, the task began when a demonstrator was allowed to eat food flavored with cocoa or cinnamon for 30 min in its own cage. Then, a demonstrator that had just eaten flavored chow was placed into the observer's cage and the two rats were allowed to interact for

30 min. All observers were tested 24 h after STFP acquisition by placing two jars filled with odorized food, and with water available. In the STFP test, one of the jars contained the chow with the flavor that was given to demonstrators (trained food) and the other jar contained different scented chow (untrained food). The observers were allowed to eat for 45 min, after which the food jars were removed and weighed to determine the amount of food eaten from each. A preference score (Percentage of trained food) for the trained odor was calculated as follows: $100 \times$(weight of trained food eaten/weight of all food eaten). Subjects' behavior during the social interaction (acquisition) and testing was recorded and the number of times each observer sniffed the muzzle, body or anogenital region of the demonstrator was scored. A sniff was defined as close orientation (<2 cm) of the observer's muzzle toward the demonstrator [47]. During the first 20 min of testing, the number of times the observer was on top of the jar with both forepaws was also scored (Jar Climbs).

Olfactory perception test. To rule out olfactory alterations due to the DCS and SCOP infusions, an additional olfactory perception test was conducted at the end of the experiment [48,49] on a sample of each group (VEH: $n = 8$, DCS: $n = 8$, SCOP: $n = 8$ and DCS+SCOP: $n = 9$). Twenty-four hours before the olfactory test, the rats were habituated to butter-flavored cookies (Brambly Hedge, Denmark). Twenty min before such habituation, they were infused with DCS or PBS and with SCOP or PBS immediately after. The rats were food-restricted for 24 hours before the test, which was conducted in clean rat cages ($50 \times 22 \times 14$-cm) and a piece of cookie was buried in one of its corners. The rats were then placed in the cage, and the latency to find the buried cookie and commence eating was timed.

Histology. Upon completion of the behavioral testing, the rats were deeply anesthetized with an overdose of sodium pentobarbital (Dolethal, Vetoquinol SA Madrid, Spain; 200 mg/kg i.p.) and perfused transcardially with 0.9% saline followed by 10% formalin. The cannulae were carefully removed and brains were postfixed in 10% formalin for at least 24 h and then submerged in a 30% sucrose solution prior to sectioning. Coronal 40-μm sections were cut on a cryostat (Shandom Cryotome FSE, Thermo Electron Corporation, Waltham, Massachusetts, USA), mounted and stained with Cresyl violet. The sections were examined under a light microscope (Olympus BX 41; Olympus Optical CO, LTD, Tokyo, Japan) and microphotographs of the cannula placements were taken using a digital camera (Olympus DP70).

Data analysis. Data from ODT were submitted to a mixed analysis of variance (ANOVA; PASW v20) in which the between-factor was Group (VEH, DCS, SCOP, DCS+SCOP) and the within-factor Session (two levels: Acquisition -the average scores for the 4 trials- and Test -the average scores for the 4 trials-). The dependent variables were Latencies and total Number of errors. Post-hoc comparisons were performed between each treatment condition and the VEH group by means of Dunnett's t-tests.

The analysis of the main dependent variable in the STFP task, Percentage of trained food, was performed by means of ANOVA with the Group factor as the independent variable (VEH, DCS, SCOP, DCS+SCOP). Post-hoc comparisons were also performed between the VEH group and the remaining groups by means of Dunnett's t-tests. In addition, a one-sample t test against a constant (50) was used for each group to determine whether the percentage of trained food eaten was different from the chance level (50%). To evaluate whether all the animals had similar opportunities of learning (similar social interaction levels), we carried out an ANOVA analysis, considering Group as the independent variable and the dependent variables were sniffs of the demonstrator's

Muzzle, sniffs of the demonstrator's Body and sniffs of the demonstrator's Anogenital region. Pearson correlation tests were used to examine the relationship between such variables and the Percentage of trained food selected. ANOVA analyses were used to analyze Total food eaten and Jar climbs that evaluated motivation to eat and explore during the 2-choice preference test. Additional mixed analyses of variance were carried out to analyze neophobia, with the dependent variables Regular food (mean g of food eaten during the last habituation session prior to training) and New food (mean g of total food eaten, trained+untrained, during the test).

Regarding the olfactory test, an additional ANOVA analysis was applied considering Group (VEH, DCS, SCOP and DCS+SCOP) as the independent variable, and Latency in finding the buried cookie as the dependent variable.

Experiment 2: Three-choice STFP

Subjects. Fifty-three male Wistar rats (mean age = 94.1 d, SD = 7.44; mean weight = 392.34 g, SD = 39.25) were used as observers and 44 rats as demonstrators (mean age = 59.02 d, SD = 6.16; mean weight 286.32 g, SD = 31.02). In experiment 2, the rats underwent surgery, microinfusion and histology using the same procedures as described for Experiment 1.

Apparatus. All observers were habituated and trained under the same conditions as in the STFP task in experiment 1 with the exception that in the habituation and test session the food tray contained three jars.

Behavioral procedure. The habituation, acquisition and testing procedures were the same as those in the STFP task from experiment 1, with the exception that, in addition to cocoa and cinnamon, 0.5% vanilla (Hacendado, Spain) was also used as a third option in the preference test.

Olfactory perception test. To rule out olfactory alterations, the same protocol as in experiment 1 was carried out on a sample of each group (VEH: $n = 9$, DCS: $n = 9$, SCOP: $n = 9$ and DCS+SCOP: $n = 8$).

Data analysis. The statistical analyses were similar to those in STFP from experiment 1, but in experiment 3 the one-sample t test was against the constant 33.3 (chance level 33.3%).

Results

Histology (Experiments 1 and 2)

When the experiments were completed, all the rats (except the demonstrators in experiments 1 and 2) were subjected to histological verification of correct bilateral cannula placements. Subjects were only included if their injector tips were located bilaterally within the PLC within the area delimited by the anterior cingulate and infralimbic cortices and in which no tissue damage resulting from the rate or volume of the infusions was detected (Fig. 1A). Specifically the cannulae were located along different brain coordinates from 3.20 to 4.20 mm anterior to bregma (Fig. 1B) according to the stereotaxic atlas [46]. Subjects with incorrectly implanted cannulae were excluded from behavioral data analyses (Experiment 1: $n = 7$, Experiment 2: $n = 7$). Thus, the final sample in experiment 1was made up of 39 subjects (ODT: VEH = 9, DCS = 10, SCOP = 9, DCS+SCOP = 11; STFP: VEH = 10, DCS = 10, SCOP = 8, DCS+SCOP = 11), and, in experiment 2, 44 subjects (VEH = 11, DCS = 11, SCOP = 11, DCS+SCOP = 11).

Behavior

Experiment 1: ODT. The analysis of Latencies (Fig. 2B) to make the correct response showed that the Group ($F_{[3,35]} = 3.81$;

$P = 0.018$), the Session ($F_{[1,35]} = 19.794$; $P < 0.0001$) and the interaction Group×Session ($F_{[3,35]} = 5.698$; $P = 0.003$) factors were statistically significant. Also, the analysis of the total Number of errors (Fig. 2C) demonstrated that the Group and Group× Session factors were statistically significant ($F_{[3,35]} = 4.896$; $P = 0.006$ and $F_{[3,35]} = 4.158$; $P = 0.013$, respectively), but not the Session factor ($F_{[1,35]} = 1.911$; $P = 0.176$). Specifically, in the acquisition session, all the groups displayed a similar performance and between-group differences were only found in the Test session. The Dunnett t-tests demonstrated statistically significant differences in Latencies and Number of errors between the VEH group and the following groups: SCOP ($P = 0.016$, $P = 0.029$, respectively) and DCS ($P = 0.05$, $P = 0.05$, respectively), but not DCS+SCOP ($P = 0.391$, $P = 0.119$, respectively).

Experiment 1: Two-choice STFP. The ANOVA analysis revealed a statistically significant effect of the Group in Percentage of trained food eaten in the test, $F_{[3,38]} = 7.588$, $P < 0.0001$ (Fig. 3B). According to the Dunnett t-tests statistically significant differences were found between the VEH group and the SCOP ($P < 0.0001$) and the DCS+SCOP ($P < 0.028$) groups, but not the DCS group ($P = 0.677$). Moreover, VEH, DCS and DCS+SCOP groups significantly performed above chance level (all t's > 5.4, all P's < 0.0001), but the SCOP group showed a performance that was not statistically different from chance level ($P = 0.098$).

The analysis of the social interaction measures (Table 1) showed no statistically significant Group effects in any of the variables (Muzzle: $F_{[3,33]} = 1.547$, $P = 0.223$; Body: $F_{[3,33]} = 1.878$, $P = 0.155$; Anogenital: $F_{[3,33]} = 0.964$, $P = 0.422$). There were no statistically significant correlations between such variables and the Percentage of trained food (Muzzle: r = −0.202, $P = 0.252$; Body: r = 0.194, $P = 0.272$; Anogenital: r = 0.251, $P = 0.152$). The analysis of the Jar climbs performed in the test (Table 1) showed that all the groups investigated both food jars to a similar degree ($F_{[3,33]} = 0.145$, $P = 0.932$) and consumed a similar amount of food ($F_{[3,38]} = 1.098$, $P = 0.363$). In the analysis of possible neophobic effects (Table 1), a mixed ANOVA analysis showed a significant effect of Food ($F_{[1,35]} = 6.085$, $P = 0.019$) but no significant effects of Group ($F_{[3,35]} = 0.268$, $P = 0.848$) or Group×Food interaction ($F_{[3,35]} = 1.784$, $P = 0.168$), thus demonstrating that, although the New food produced a certain neophobic response, the pattern of consumption was similar for all groups.

Experiment 1: Olfactory perception test. The performance in both tasks did not seem to be related to changes in olfactory sensitivity (Table 2) since no statistically significant between-group differences were observed when the Latency to find a buried sweet-smelling cookie was analyzed 24 h after injections ($F_{[3,32]} = 0.756$, $P = 0.528$).

Experiment 2: Three-choice STFP. The main analysis revealed a significant effect of the Group in Percentage of trained food eaten in the test ($F_{[3,43]} = 3.395$ $P = 0.027$) (Fig. 3C). The contrast analyses showed that the preference score of the VEH group was not statistically different from that of DCS and DCS+SCOP groups ($P = 0.935$ and $P = 0.929$, respectively), and tended to be statistically higher than the SCOP group ($P = 0.06$) score. Similarly to experiment 1, and confirming the latter analysis, the VEH, DCS and DCS+SCOP rats significantly performed above chance level (all t's > 2.6, all P's < 0.025), whereas the SCOP rats showed a performance not significantly different to chance ($P = 0.505$). There were no statistically significant Group effects in any of the variables measured during the social interaction (Table 3) (Muzzle: $F_{[3,43]} = 1.947$, $P = 0.138$); Body: $F_{[3,43]} = 1.744$, $P = 0.173$; Anogenital: $F_{[3,43]} = 2.186$, $P = 0.105$). No statistically significant correlations were found between these variables and the Percentage of trained food (Muzzle: r = −0.069, $P = 0.664$; Body: r = 0.260, $P = 0.096$; Anogenital: r = 0.055, $P = 0.728$). No statistically significant between-group differences were observed either in the total amount of food consumed during the test ($F_{[3,43]} = 1.664$, $P = 0.190$) or in the Jar climbs (Table 3) ($F_{[3,43]} = 1.356$, $P = 0.274$). Mixed ANOVA analysis did not show any significant effect of Food ($F_{[1,40]} = 1.214$, $P = 0.277$), Group ($F_{[3,40]} = 1.717$, $P = 0.179$) or Group×Food interaction ($F_{[3,40]} = 0.813$, $P = 0.317$) (Table 3), demonstrating that SCOP or DCS did not produce neophobic reactions.

Experiment 2: Olfactory perception test. Performance in the 3-choice STFP test did not seem to be related to deficits in olfactory sensitivity (Table 2) since no statistically significant between-group differences were observed in the test ($F_{[3,34]} = 0.553$, $P = 0.650$).

Table 2. Olfactory perception test.

	Experiment 1	Experiment 2
VEH	30.13±11.96	24.67±13.5
DCS	24.56±10.11	25.56±10.45
SCOP	32.25±12.88	25.33±11.18
DCS+SCOP	28.15±11.64	31.75±15.63

Means ± SD of the latency (sec) to find a buried cookie in the olfactory perception test carried out in experiments 1 and 2.
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Table 1. Ancillary variables measured in STFP task in experiment 1.

	Habituation	Social Interaction			2-choice test	
	Regular food	Muzzle	Body	Anogenital	Jar climbs	Total (new) food
VEH	8.93±4.96	44.60±8.95	67.70±10.58	27.70±6.68	64.80±17.73	7.67±2.12
DCS	10.73±3.9	39.22±11.3	54.33±11.31	30.22±7.36	72.22±30.77	7.53±2.71
SCOP	8.59±2.23	34.75±8.26	68.00±17.78	26.75±6.75	68.00±14.17	8.19±2.36
DCS+SCOP	9.18±2.86	44.82±9.30	56.82±18.65	24.91±7.11	70.82±32.17	9.5±3.58

Means ± SD of the amount of regular food consumed during the last rehabituation (unodorized ground food); Means and ± SD of the number of sniffs scored during the social interaction; Means and ± SD of the number of jar climbs during the first 20 min of the 2-choice STFP test; Means and ± SD of the total amount of total odorized food eaten during the test (new food, -trained+untrained-).
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Table 3. Ancillary variables measured in STFP task in experiment 2.

| | Habituation | Social Interaction | | | 3-choice test | |
	Regular food	Muzzle	Body	Anogenital	Jar climbs	Total (new) food
VEH	11.04±4.67	35.20±10.1	41.00±10.31	25.90±11.0	82.12±30.87	9.69±1.76
DCS	8.78±3.75	42.36±22.8	54.55±26.20	28.27±12.4	69.38±25.38	8.8±2.62
SCOP	7.99±4.39	54.4±26.24	51.10±15.27	39.3±22.13	71.91±32.34	7.22±2.71
DCS+SCOP	8.13±3.92	52.27±22.4	58.55±22.47	42.55±22.6	55.33±19.72	7.87±3.68

Means ± SD of the amount of regular food consumed during the last rehabituation (unodorized ground food); Means and ± SD of the number of sniffs scored during the social interaction; Means and ± SD of the number of jar climbs during the first 20 min of the 3-choice STFP test; Means and ± SD of the total amount of total odorized food eaten during the test (new food, -trained+untrained-).

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Discussion

The current research shows that potentiating NMDAR function in the PLC by DCS may attenuate mnemonic deficits induced by muscarinic receptor antagonism in two olfactory learning paradigms, a stimulus-reward task and a relational memory task [38,50,51], which share some underlying structures, such as the PLC, but not others, such as the hippocampus [28,33,34]. Such findings cannot be attributed to alterations in olfactory perception, social investigation, neophobic responses or motor activity since DCS and SCOP infusions, alone or in combination, did not show any effect in the olfactory sensitivity test or the ancillary variables scored during social interaction and food preference testing. Likewise, the counteraction of SCOP-induced deficits was observed in other learning paradigms (see Introduction section) using DCS administration, which also attenuated mnemonic deficits induced by the blockade of other neurotransmission systems, such as NMDA [52]. Moreover, DCS has been able to revert memory deficits associated to aging [53,54], stress [55], traumatic brain injury and hipocampal or medial septal lesions [56,57,58,59].

In the present experiments, the reversion effect of the pre-training DCS treatment was highly noticeable on the ODT in which the DCS+SCOP group performance in the 24 h drug-free test did not significantly differ from that of the VEH, in contrast to the poorer performance by the SCOP rats in terms of both latencies and errors. This agrees with previous findings demonstrating that pre-acquisition intra-PLC DCS rescued ODT memory impairment induced by parafascicular lesions [32]. As for the STFP, DCS microinfusion also ameliorated the SCOP-induced deficits since DCS+SCOP rats showed a preference score superior to the chance level, like the VEH and DCS rats and unlike the SCOP rats which performed around 50% (2-choice test) or 33.3% (3-choice test). Nonetheless, DCS appeared to be more effective in the 3-choice version of the task because the DCS+SCOP group did not significantly differ from the VEH group, in contrast to the 2-choice paradigm. A possible explanation for such an outcome is that the prefrontal cortex may be more actively engaged in the STFP task when its difficulty is increased and decision-making is arduous [42,43], which would agree with the proposed role of the prefrontal cortex in a variety of processes associated with executive function, including decision-making [60]. This would suggest that challenging tests (e.g. involving several choice alternatives) may be a more appropriate way to evaluate promnesic effects [61].

The data presented here also show that a single injection of DCS in the PLC prior to learning improved the odor-reward task in SCOP-untreated rats, as the group treated with DCS alone performed significantly better than the VEH group. This effect replicates previous findings indicating that DCS-treated rats committed significantly fewer errors in a 24 h ODT test [31] and corroborates that NMDARs in the PLC modulate ODT memory formation since microinfusion of the NMDAR antagonist APV into the PLC (but not the hippocampus) impaired an ODT retention test [35]. Although the outcome of DCS only affecting the 24 h test, as opposed to acquisition, may be unexpected, it rules out the possibility of a state-dependent learning situation. Moreover, it has previously been shown that PLC SCOP infusions or thalamic lesions carried out prior to ODT acquisition may result in delayed effects [29,49]. As for the STFP task, involvement of the NMDA receptors has previously been demonstrated in experiments administering NMDAR antagonists systemically or in the hippocampus, inducing amnesic effects [62,63]. In contrast, and also in opposition to the ODT results, our research shows that the positive modulation of PLC NMDAR did not produce any significant effect in social memories transmitted by odorous stimuli in SCOP-free rats.

Such findings suggest that DCS may have differential effects depending on the nature of the learning paradigm and may be interpreted as DCS enhancing implicit or procedural tasks, such as ODT, but its facilitative influence on relational paradigms, such as STFP, was limited. In this regard, there is evidence showing, on the one hand, that DCS facilitated ODT [15], conditioned fear responses [64,65,66], conditioned flavor-taste preference and conditioned-taste aversion [13,67], or procedural learning in humans [68]. On the other hand, no facilitative effects of DCS administration were found in the retention of Morris water maze (MWM) learning in rodents [69,70], or declarative word-pair learning in humans [68]. Nevertheless, other reports point to the facilitation of hippocampal-dependent paradigms, such as MWM [11,53,71], radial arm maze [72], linear maze [73], object-location [74], trace eye blink conditioning [75], an episodic-like memory task [61] and item-category associations [76]. Indeed, the view that distinguishes declarative/hippocampal tasks from procedural/non hippocampal tasks has been challenged and it has been suggested that multiple brain regions involved in learning are linked to each other in a coordinated way, rather than working in isolation and competing for control over behavioral output [77].

The inconsistent effects of DCS on learning and memory may be attributable to additional factors observed in the different experiments, such as dissimilar drug doses and injection timings, test protocols, rat strains or species, and/or ages. In view of such evidence, our results may potentially contain some limitations in the STFP task. The DCS dosage, for example, may not have been optimal, which is an important factor in that the therapeutic window for DCS to enhance human fear memory extinction has

been reported as narrow [78]. In this respect, although the previous studies testing intracerebral DCS administration used the same dosage (10 µg/site) [15,31,79,80,81], higher doses might have been more appropriate to find enhancing effects in VEH rats. Indeed, it has been reported that only a higher dose of systemic DCS was able to promote episodic-like memory [61], although lower doses potentiated memory in non-relational aversive paradigms [82]. However, the use of higher doses of DCS could not induce outstanding facilitative effects since a reversed U-shaped dose-response curve has been described in behavioral and electrophysiology studies [83,84,85]. Additionally, other brain areas besides the PLC may be more sensitive to intracerebral DCS administration, such as the hippocampal formation, which has been clearly involved in the consolidation of STFP [33,38] and other relational tasks.

The present study also confirms that the blockade of cholinergic muscarinic receptors in the PLC notably damaged memory in ODT and STFP. Such findings corroborate previous data showing that intra-PLC post-training SCOP infusions disrupted memory tests performed one day after ODT or STFP acquisition [27,29,28]. Additional examples of SCOP-induced deficits can be found when the drug is injected in other brain regions, e.g. the basolateral amygdala, which also interrupted STFP [37], the hippocampus impairing contextual fear conditioning [86], the cingulated and insular cortices disrupting inhibitory avoidance [87,88] or the perirhinal cortex decreasing recognition memory [89]. Although the administration of muscarinic receptor antagonists has frequently been considered a pharmacological model for cholinergic cognitive impairment mimicking some of the features of neurodegenerative disorders [90], the use of SCOP remains controversial due to its wide mode of action and spectrum of behavioral effects [91]. In this respect, it has been suggested that selective M1 antagonists may constitute a relatively more valid pharmacological model of cognitive impairment as they are likely to affect cognitive function in a relatively more specific manner [90]. Nevertheless, importantly to the present research, the fact that SCOP impairs social memory [27,28,92], combined with the clinical observation of reduced social contacts in dementia patients, may suggest that social behavior based-tasks that are sensitive to muscarinic blockade, such as the STFP, may offer a relevant approach with translational value for experimental models of cognitive dysfunction.

As for the mechanisms of action, DCS effects have been interpreted in terms of synaptic plasticity modulation [93,94], considering that it is capable of enhancing NMDAR-dependent synaptic potentials and LTP in the CA1 hippocampal field of control adult and old rats [95,96,97]. Similarly, DCS reinstated hippocampal LTP and improved neurological and learning recovery in brain-damaged mice [59] and neural cell adhesion molecule-deficient mice [98]. It may be complex, however, to understand why the combination of DCS and SCOP, with

different pharmacological mechanisms, demonstrated a balancing or compensatory effect. Some data indicate that cholinergic actions may be mediated via the regulation of NMDARs, whose properties enable many forms of indirect modulation [99]. In particular, the stimulation of muscarinic receptors is known to facilitate the activation of NMDARs causing a long-lasting facilitation of excitatory postsynaptic potentials [2]. Also, a recent study has shown that the synergistic coactivation of muscarinic and glutamatergic receptors is essential for long-lasting LTP and that cooperation between such receptors is needed to induce BDNF-dependent long-lasting memory storage [100]. Most of these actions have been described in the hippocampal region, although they may also take place in neocortical regions such as the medial prefrontal cortex [101,102]. In this context, it has been suggested that cholinergic and NMDA receptors jointly modulate the electrophysiological functioning of cortical cells [54,103]. Thus, the activation of muscarinic receptors has been reported to increase glutamate release, which positively modulates neuronal activity in cortical pyramidal cells [104,105].

Therefore, although our results do not fully demonstrate an interactive relationship between the glutamate and acetylcholine systems in learning and memory modulation, they are in line with other studies suggesting such a relationship (see Introduction section). Consequently, in the present experiments, SCOP may have disrupted potential plasticity mechanisms [106] in the PLC, which were possibly restored by DCS administration, and thus improved ODT and STFP memory. Although such tasks are based on olfactory cues, similar effects may well be found in mnemonic tasks depending on different sensory modalities. This is suggested by the fact that the PLC has been related, for instance, to the reversal learning of associative visual discrimination tasks [107]. This would also indicate that the PLC not only participates in specific associative memory but also in more general aspects of cognitive demand, such as behavioral flexibility, which may be important in processing information for different kinds of memory [108]. Further research would also need to be performed in order to determine the precise mechanisms underlying the interactive process between neurotransmitter systems and the most effective doses and sites of action of DCS to facilitate different memory paradigms and thus contribute to accelerating the effectiveness of cognition-enhancing therapies.

Acknowledgments

Author Contributions

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3.   Experiments 5 and 6: Efectes de la DCS a l'HPC per revertir dèficits produïts per l'SCOP en la TSPA i la plasticitat neural

D-cycloserine prevents relational memory deficits and suppression of long-term potentiation induced by scopolamine in the hippocampus

3.1   Theoretical approach

As suggested in the previous study (Portero-Tresserra et al. 2013), an interactive relationship between NMDA and muscarinic transmission has been proposed in learning and memory modulation, and one of the brain regions where such interaction may take place is the PLC. Nevertheless, few studies have evaluated the specific brain areas where such interaction may occur. The hippocampus is an important structure involved in memory formation and synaptic plasticity processes which depend on glutamatergic and cholinergic pathways (Power et al. 2003). Thus, administration of muscarinic or NMDAR antagonists, such as SCOP or APV, into the dorsal or ventral hippocampus produced memory deficits in olfactory relational paradigms and spatial learning (Carballo-Marquez 2009; Farr et al. 2000; Brun et al. 2001). Moreover, NMDAR and cholinergic transmission seem to interact in this region since hipocampal cholinergic actions may be mediated via the regulation of NMDAR, whose properties enable many forms of indirect modulation (Collingridge 2013). Specifically, a synergistic coactivation of muscarininc and NMDAR in the hippocampus seems to be necessary for long term potentiation (LTP), probably due to the fact that the stimulation of muscarinic receptors facilitates NMDAR activation (Navakkode & Korte 2012). In addition, such relation has also been found in behavioral experiments in which concomitant administration of subthreshold doses of SCOP and NMDA antagonists into CA1 induced amnesia in spatial learning (Khakpai et al. 2012; Figueredo et al. 2008).

Early research also suggested that the administration of DCS into the dorsal hippocampus may compensate SCOP-induced impairments in spatial memory (Ohno & Watanabe 1996; Kishi et al. 1998). Nevertheless, the underlying mechanism by which this reversal effect may occur needs further investigation. It has been described that DCS facilitated NMDA-dependent synaptic plasticity in CA1 (Billard & Rouaud 2007) and improved LTP deficits in brain-damaged animal models (Yaka et al. 2007). Moreover, it has also been found that SCOP administration impaired LTP in CA1 region of the hippocampus (Lin et al. 2004). Thus, one possibility to consider is that DCS may enhance memory by modifications in synaptic plasticity which could be impaired by SCOP.

In this context, the hypothesis of the present study was that glutamatergic and cholinergic transmission interacts in the hippocampus to modulate relational memory and synaptic plasticity mechanisms. Hence, intra hipocampal administration of DCS and SCOP was applied in two different experiments, using behavioral and electrophysiological procedures.

- Experiment 5: The main objective was to explore the ability of DCS administered in the ventral hippocampus (vHPC), in compensating the dysfunction of muscarinic receptors in a relational learning task. Thus, the DCS was infused 20 minutes before the STFP acquisition, SCOP immediately afterwards, and memory was measured in a standard 2-choice STFP retention test 24 hours after acquisition.

- Experiment 6: The administration of DCS in hippocampal slices was carried out in order to rescue possible SCOP-induced deficits in hippocampal LTP. To that end, electrophysiological recordings in CA1 synapses were performed, under the influence of DCS and SCOP perfusions.
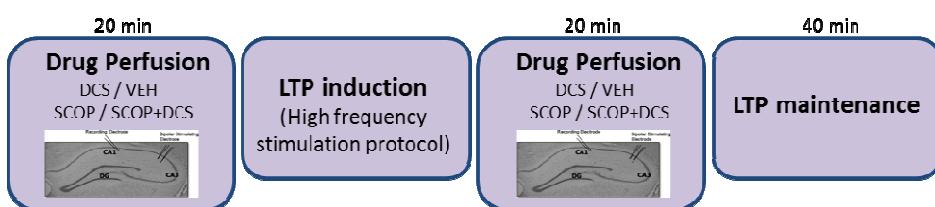
3.2   Experiment 5

3.2.1   Methods and procedure (Figure 25)

In order to test whether DCS may compensate the behavioral deficits induced by SCOP, 20 minutes before STFP acquisition the rats received bilateral intra-vHPC infusions of DCS (10µg) or VEH, and immediately afterwards intra-vHPC SCOP (40µg) or VEH. Regarding the DCS dosage, it has been based on previous research with intracerebral infusions (Akirav et al. 2009). The dose of SCOP was higher than the used in a preceding study in which the 20µg did not produce a complete amnesia (Carballo-Marquez 2009). Finally, 24 hours later, the animals carried out the STFP memory retention test.

The final sample of this experiment was made up of 42 subjects distributed into the following groups: VEH (n=10), DCS (n=10), SCOP (n=12) and DCS+SCOP (n=10). All the procedures were similar to those used in the experiment 3, with the exception of that they underwent sterotaxic implantation of bilateral chronic guide cannulae in the vHPC.
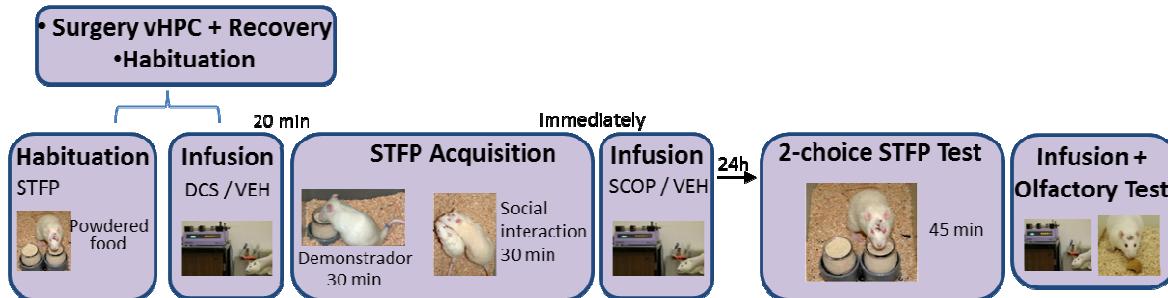
Figure 25. The behavioral procedure used for the experiment 5

3.2.2   Summary of the main results

- The NMDAR co-agonist DCS administered into the vHPC prior to the STFP acquisition rescued memory impairment induced by SCOP. The group DCS+SCOP showed higher preference than the SCOP group in the 24-hour retention test, performing not differently from the VEH group.
- The administration of DCS alone into the vHPC prior to acquisition did not produce positive effects on the STFP retention test since DCS group did not differ from the VEH group.
- The intra-vHPC injection of SCOP after the STFP acquisition induced marked memory impairment on the 24-hour retention test, inducing a preference score around chance level.

- The infusion of DCS and SCOP did not produce any effect on olfactory perception, social investigation, neophobic responses or motor activity since all the animals demonstrated similar latency to find a buried cookie and similar performance in the ancillary variables scored during social interaction and food preference testing.

3.3   Experiment 6

3.3.1   Methods and procedure (Figure 26)

The proposal of this experiment was to explore whether DCS (50 µM and 100µM) may rescue putative deficits induced by SCOP (100µM) in the induction/maintenance of LTP. To that end, electrophysiological recordings in CA1 synapses were analyzed under the influence of DCS and SCOP perfusions (alone and in combination) in hippocampal slices. The drugs dosage was based on previous electrophysiological studies that have found facilitative effects of DCS (Rouaud & Billard 2003) and LTP impairment produced by SCOP (Ye et al. 2001).

Electrophysiological assays were carried out in a final sample of 40 hippocampal slices from Wistar rats which were distributed among the different experimental groups: DCS 50µM (n=5), DCS 100µM (n=4) VEH group (n=7), SCOP 100µM (n=8), DCS 50µM+SCOP 100µM (n=5) and DCS 100µM+SCOP 100µM (n=4). At the beginning of the experiment, the animal's brains were removed and transverse hippocampal slices were cut and incubated during 2 hours in a ringer solution. Afterwards, field excitatory postsynaptic potentials (fEPSP's) evoked by stimulating Schaffer collateral fibers were recorded in hippocampal CA1 during 100 minutes. The synaptic strength was assessed by measuring the initial slope of the fEPSP. During the first 20 minutes of the recording, a baseline period with stable I-O responses was carried out, after which the different pharmacological treatments were perfused during 40 min. Following 20 minutes of drug perfusion, Schaffer collateral fibers were tetanized with three 100-Hz pulses of 100 µs/sec duration every 20 seconds (high frequency stimulation, HFS) to induce saturated-LTP. Following LTP induction, recording was registered during 60 min, 20 minutes with drug and 40 minutes with KRB solution alone, to analyze the LTP maintenance.
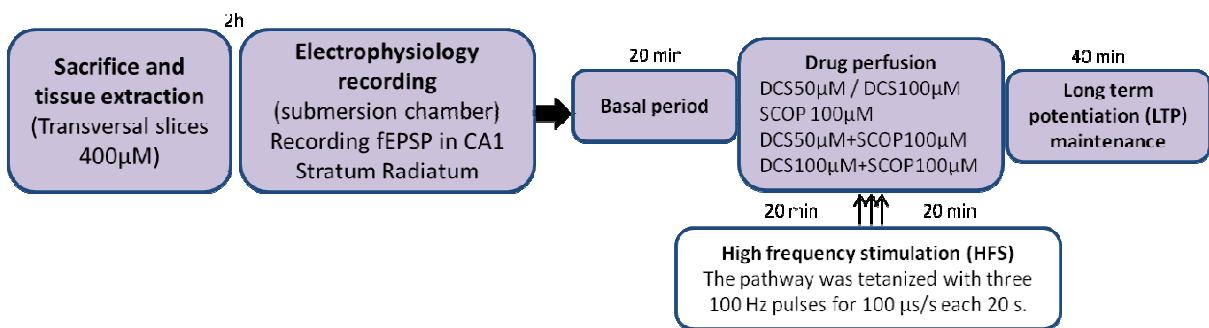
Figure 26. The behavioral procedure used for the experiment 6

3.3.2 Summary of the main results

- The perfusions of SCOP into the hippocampus suppressed LTP maintenance. In the last 10 minutes of recording SCOP group showed lower percentage of potentiation compared to VEH group.

- The administration of two doses of DCS into the hippocampus prior to the LTP induction increased the magnitude of the fEPSPs but did not produce effects on the LTP maintenance. Thus, on the last 10 minutes of the recording the DCS groups did not differ from the VEH group.

- The perfusion of two different doses of DCS into the hippocampus restored the LTP maintenance impairment due to muscarinic blockade. On the last 10 minutes of the recording, both DCS+SCOP groups presented higher fEPSPs than the SCOP group that were similar to those in the VEH groups.

D-cycloserine prevents relational memory deficits and suppression of long-term potentiation induced by scopolamine in the hippocampus

ABSTRACT

Previous research has demonstrated that systemic D-cycloserine (DCS), a partial agonist of N-methyl-D-aspartate receptor (NMDAR), enhances memory processes in different learning paradigms and reverses mnemonic deficits produced by diverse manipulations, such as muscarinic receptors blockade. In the present study two experiments were conducted in rats to investigate whether DCS administered in the hippocampus may rescue social memory deficits, induced by injection of scopolamine (SCOP), through enhancing synaptic plasticity. In experiment 1, we assessed whether DCS would prevent SCOP-induced amnesia on a relational olfactory learning paradigm that requires the integrity of the cholinergic system, the social transmission of food preference (STFP). The results showed that DCS (10 g / site) injected into the vHPC before STFP acquisition compensated the retention deficit elicited by post training intra-vHPC SCOP (40µg), although DCS alone did not improve memory. In experiment 2, we evaluated whether the perfusion of DCS in hippocampal slices may potentiate synaptic plasticity and thus recover the SCOP-induced deficits in long-term potentiation (LTP). The findings showed that DCS (50µM and 100µM) was able to rescue SCOP (100µM)-induced LTP maintenance impairment. In addition, DCS alone (50 µM and 100 µM) did not significantly potentiated LTP maintenance, although it enhanced field excitatory postsynaptic potentials before high frequency stimulation (HFS). Such results suggest that positive modulation of the NMDAR, by activation of the glycine-binding site, may compensate relational memory impairments due to hippocampal muscarinic neurotransmission dysfunction possibly through enhancements in LTP maintenance.

INTRODUCTION

One of the most relevant targets of neuroenhancement strategies is the N-methyl-D-aspartate receptor (NMDAR), which is the predominant site for inducing learning-related synaptic plasticity in the hippocampus (Lee and Silva, 2009; Li and Tsien, 2009). D-cycloserine (DCS), a partial agonist of the NMDAR that binds to the glycine-site, enhances receptor activation in the presence of glutamate (Norberg et al. 2008), and recently has gained substantial attention for its potential in facilitating different cognitive processes. Studies in rodents indicate that DCS, administered systemically or

intracerebrally, improves learning and memory processes, including acquisition, consolidation, reconsolidation and extinction, in several paradigms such as socially reinforced learning, odor discrimination, fear conditioning and others (Lelong et al., 2001; Walker et al., 2002; Davis et al., 2006; Golden and Houpt, 2007; Zlomuzica et al., 2007; Lee et al., 2009; Villarejo-Rodríguez et al., 2010; Modi and Young, 2011; Otto 2011; Rodgers et al., 2011; Portero-Tresserra et al., 2013). Evidence from studies in humans suggest that DCS promotes both the consolidation and extinction of conditioned fear (Norberg et al., 2008b; Kalisch et al., 2009) and also enhances declarative learning (Onur et al., 2010).

It has also been reported that DCS attenuates mnemonic deficits induced by several factors, such as aging (Baxter et al., 1994; Aura and Riekkinen, 2000), stress (Yamamoto et al., 2008), traumatic brain injury and hippocampal, medial septal or thalamic lesions (Schuster and Schmidt, 1992; Temple and Hamm, 1996; Jr et al., 1998; Yaka et al., 2007; Adeleye et al., 2010; Villarejo-Rodríguez et al., 2013) and improves cognition in Alzheimer's disease patients (Tsai et al., 1999). DCS also ameliorates memory impairments produced by manipulations of neurotransmitter systems, such as glutamatergic (Kawabe et al., 1998) or cholinergic blockade. In particular, the acute administration of DCS reverses learning and memory failure in rats treated with the muscarinic acetylcholine (ACh) receptor antagonist scopolamine (SCOP), which may be considered a pharmacological model for cholinergic cognitive impairment (Klinkenberg and Blokland, 2010). Thus, systemic pre-learning DCS infusion attenuated SCOP-induced deficits in acquisition of spatial tasks (Sirviö et al., 1992; Fishkin et al., 1993; Pitkänen, Sirviö, MacDonald, Niemi, et al., 1995; Puumala et al., 1998), brightness discrimination (Andersen et al., 2002) and visual recognition (Matsuoka and Aigner, 1996a). It has also been shown that DCS antagonized memory decreases produced by SCOP in young and elderly healthy volunteers (Jones et al., 1991). Such results point to an interactive relationship between the NMDA and the muscarinic transmission in learning and memory modulation, also suggested by additional pharmacological (Matsuoka and Aigner, 1996b; Ohno and Watanabe, 1996; Li et al., 1997; Hlinák and Krejcí, 1998; Figueredo et al., 2008) and electrophysiological experiments (Markram and Segal, 1990; Drever et al., 2007).

However, few studies have assessed the brain regions where such an interaction may take place (Mahmoodi et al., 2010; Khakpai et al., 2012) or the function of intracerebral DCS as a preventive treatment for deficits produced by cholinergic deficiency. Early research showed that administration of DCS into the dorsal hippocampus ameliorated SCOP-induced spatial working memory impairment (Ohno and Watanabe, 1996; Kishi et al., 1998). Moreover, a recent study demonstrated that DCS in the prelimbic cortex (PLC) reverted SCOP-induced deficits in olfactory learning tasks involving stimuli discrimination and socially-guided behavior (Portero-Tresserra et al., 2013b). Although beneficial effects of DCS have been described in SCOP-induced behavioral memory deficits, the physiological mechanisms by which this may occur are poorly investigated. On the one hand, it has been suggested that DCS may enhance memory facilitating NMDAR-dependent synaptic

potentials and synaptic plasticity in the CA1 hippocampal field (Rouaud and Billard, 2003; Billard and Rouaud, 2007). Accordingly, DCS reinstated long-term potentiation (LTP) in CA1 and improved memory and neurological impairments in both brain-damaged mice (Yaka et al., 2007) and neural cell adhesion molecule-deficient mice (Kochlamazashvili et al., 2012). On the other hand, SCOP administration mpaired LTP in the CA1 (Hirotsu et al., 1989; Calabresi et al., 1999; Ye et al., 2001; Lin et al., 2004; Sánchez et al., 2009) and altered hippocampal glutamate receptor levels (Falsafi et al., 2012).

In this context, the aim of the present study was to assess whether intra-hippocampal DCS may prevent memory deficits elicited by SCOP in the social transmission of food preference (STFP) task, as well as to explore if DCS may rescue possible SCOP-induced deficits in hippocampal LTP, which, as far as we know, has not been investigated yet. Thus, in the first experiment, we sought to confirm whether DCS, as it has been reported with intra-PLC administration (Portero-Treserra et al., 2013b), reverses SCOP-induced deficits in the STFP, which is a paradigm of social learning or "learning from others", involving an ethologically meaningful test of olfactory memory (Galef and Wigmore, 1983). STFP is considered a hippocampal-dependent task with no explicit contextual or spatial memory component that entails many features of relational memory (Bunsey and Eichenbaum, 1995; Eichenbaum, 1999, 2000; Alvarez et al., 2001, 2002) and that requires the integrity of the cholinergic system (Berger-Sweeney et al., 2000; Ross et al., 2005), specifically in the hippocampus (Vale-Martinez et al., 2002). In the present study, an acute pre-learning DCS treatment, similar to that used in previous experiments (Villarejo-Rodríguez et al. 2010, 2013; Portero-Tresserra et al. 2013a), was applied in the ventral portion of the hippocampus (10 µg/site) since muscarinic receptors of this region contribute to a certain extent to STFP memory consolidation (Carballo-Marquez et al., 2007). Moreover, enhanced c-fos activity (Countryman, Kaban, et al., 2005; Ross and Eichenbaum, 2006; Smith et al., 2007) and CREB phosphorylation (Countryman, Orlowski, et al., 2005) have been found in the ventral hippocampus (vHPC) at different phases of STFP. In the second experiment, we assessed whether two doses of DCS (50 µM and 100µM) may rescue putative deficits induced by SCOP in the induction/maintenance of LTP, considered a basic neuronal activity underlying memory formation (Bliss and Collingridge, 1993). To that end, electrophysiological recordings in CA1 synapses were carried out under the influence of DCS and SCOP perfusions (alone and in combination) in hippocampal slices.

MATERIALS AND METHODS

Experiment 1

Subjects

Fifty male Wistar rats, obtained from our laboratory breeding stock, (Prolabor, Charles River Laboratories, Arbresle, France) with a mean age of 94.53 days (SD=6.231) and a mean weight of 402.795 g (SD=33.86) at the beginning of the experiment were used as observer subjects. An

additional set of 36 male Wistar rats (mean age=57.25 d, SD=4.89; mean weight 225.17 g, SD=47.55) at the beginning of the experiment, served as demonstrator subjects. Juvenile demonstrator rats were used in order to avoid fighting and favor social interaction (Alvarez et al., 2002; Vale-Martinez et al., 2002).

Throughout the experiment the subjects were singly housed in 50x22x14-cm plastic-bottomed sawdust-bedded cages. All rats were maintained in a humidity and temperature-controlled environment on a 12-hour light-dark cycle. Experiments were performed during the light phase. Rat-chow pellets (Scientific Animal Food and Engineering, Augy, France) and water were provided ad libitum except during habituation, acquisition and test sessions. In such phases, the rats were submitted to a food restriction schedule (12 g/d for observers to maintain body weight at 85% of freely feeding weight, and 10g/d for demonstrators). Every day the animals were weighed, handled for 5 min and the observers were restrained for 2 min in order to habituate them to the injection procedure. All procedures were carried out in compliance with the European Community Council Directive for care and use of laboratory animals (86/609/ECC) and with the Generalitat de Catalunya authorization (DOGC 2450 7/8/1997, DARP protocol number 5959).

Surgery

Observers were anesthetized with isoflurane and underwent stereotaxic implantation of bilateral chronic guide cannulae in the vHPC following procedures explained in detail elsewhere (Carballo-Marquez et al., 2009). Each guide cannula comprised one 26-gauge metal tube projecting 7 mm from the pedestal (Plastics One, Bilaney Consultants GMBH, Düsseldorf, Germany). The stereotaxic coordinates for the vHPC implantation were: AP, -5.0mm; ML, ±5.0 mm; and DV, -6.8 mm (Paxinos and Watson, 1997). Sterile dummy stylets (Plastics One) were placed into the cannulae to prevent occlusion. After surgery, rats were returned to home cages for 10 days (four for recovery, four for food restriction and two for rehabituation to ground food) before behavioral training. During the 10-day recovery period, the dummy stylets were changed every other day.

Microinfusion procedure

The rats received the drug infusions 20 min before STFP acquisition (DCS/PBS) and immediately after (SCOP/PBS). To carry out this procedure, the rats were gently restrained while the dummy stylets were removed and replaced with a 33-gauge stainless-steel injector (Plastics One) extending 1mm below the cannula tips. The injectors were connected by polyethylene tubing (Plastics One) to two 10-µl syringes (SGE Analytical Science, Cromlab S.L. Barcelona, Spain) mounted in an infusion pump (11 Plus Syringe Pump, Harvard Apparatus Inc., Holliston, Massachusetts, USA). DCS (Sigma-Aldrich, Madrid, Spain) and SCOP (Scopolamine Hydrobromide USP, Sigma–Aldrich, Madrid, Spain) were dissolved in PBS (phosphate-buffered saline 0.1M, pH 7.4) and dose of 10µg/hemisphere (DCS) and 40µg/hemisphere (SCOP) were administered to rats. The rats in the control VEH groups received PBS injections. The solutions were infused bilaterally in a volume of 0.5 µl / hemisphere for 2 min. The inner cannulae were left in place for 1min after the infusion was

complete to allow for diffusion. The concentration and volume of the DCS was based on a previous study in which intracerebral DCS reversed behavioral deficits associated to SCOP infusions (Portero-Tresserra et al. 2013b) and additional studies indicating cognition enhancing properties (Villarejo-Rodríguez et al., 2010; Portero-Tresserra et al., 2013). The SCOP dose was chosen based on precedent findings showing that intra vHPC injections of 20μg SCOP did not completely obliterate STFP memory (Carballo-Marquez et al., 2009). Moreover, a pilot study showed that 40μg of SCOP disrupted memory consolidation without inducing motor alterations.

Behavioral procedure

Habituation to food jars

The apparatus for STFP habituation, acquisition and testing is described in more detail in Portero-Tresserra et al., (2013b). After seven days of food restriction and prior to surgery, observers and demonstrators were habituated during three days to powdered chow (Scientific Animal Food and Engineering, Augy, France). A similar procedure was repeated 7 d after surgery for the observers (two 45-min rehabituation sessions). Subsequently, animals were food-restricted once again for two days before the training–testing sessions began.

STFP acquisition and test

The STFP training followed procedures explained elsewhere (Boix-Trelis et al., 2007). Essentially, the task began when a demonstrator was allowed to eat food flavored with 2.2% cocoa (Oxfam Fairtrade, Gent, Belgium) or 1% cinnamon (Carmencita, Alicante, Spain) for 30 min. Following the 30-min period, a demonstrator that had just eaten flavored chow was placed into the observer's cage. The two rats were allowed to interact for 30 min. All observers were tested 24 h after acquisition, based on previous studies showing amnesic effects after such timing (Carballo-Marquez et al., 2009). In the test, two jars were filled with odorized food, one contained the chow given to demonstrators (trained food) and the other a different scented chow (untrained food). The observers were allowed to eat for 45 min, after which both food jars were removed and weighed to determine the amount of food eaten from each. A preference score (Percentage of trained food) for the trained odor was calculated as follows: 100 x (weight of trained food eaten/weight of all food eaten).

Subjects' behavior during the social interaction and testing was recorded using a video camera (JVC, Everio Model GZ-X900) connected to a monitor. We scored the number of times each observer sniffed the muzzle, body or anogenital region of the demonstrator. A sniff was defined as close orientation (<2 cm) of the observer's muzzle toward the demonstrator (Wrenn et al., 2003). Fighting and grooming during the social interaction were also scored. During the first 20 min of testing, the number of times the observer was on top of the jar with both forepaws was also scored (Jar climbs). To determine whether drugs infusion produced changes in neophobia, we compared the amount of Regular Food eaten during the last post-surgery habituation (unodorized ground food) and the amount of new food eaten during the test (total odorized food, trained + untrained). To rule out olfactory

alterations due to the DCS and SCOP infusions, an additional olfactory perception test (described in Portero-Tresserra et al., 2013b) was conducted at the end of the experiment.

Histology

Upon completion of the behavioral study, the rats were deeply anesthetized with an overdose of sodium pentobarbital (Dolethal, 200 mg/kg; Vetoquinol S.A., Madrid, Spain) and perfused transcardially with PBS (pH: 7.4) followed by 4% paraformaldheyde in 0.1 M PBS at a flow rate of 40 ml/min. Subsequently, the cannulae were carefully removed and brains were postfixed in paraformaldehyde for two hours and then submerged in a 20% sucrose solution prior to sectioning. Coronal 40-µm sections were cut on a cryostat (Shandom Cryotome FSE, Thermo Electron Corporation, Massachusetts, USA), mounted and processed for acetylcholinesterase histochemistry, essentially as described elsewhere (Paxinos and Watson, 1997). The sections were examined to verify cannulae placement under a light microscope (Olympus BX 41; Olympus Optical CO, LTD, Japan). Microphotographs of the cannulae placements were obtained using a digital camera (Olympus DP70).

Data analysis

The main analysis of the variable percentage of trained food was performed by means of ANOVA (PASW v20) with the group factor as the independent variable (VEH, DCS, SCOP and DCS+SCOP). Specific between-group comparisons were performed and the Bonferroni correction was used. In addition, a one-sample t test against a constant (50) was used for each group to determine whether the percentage of trained food eaten was different from the chance level (50%).

To evaluate whether all animals had similar opportunities of learning (similar social interaction levels), we carried out ANOVA analyses, considering group as the independent variable and the dependent variables were sniffs of demonstrator's muzzle, sniffs of demonstrator's body, sniffs of demonstrator's anogenital region, fighting and grooming. Pearson correlation tests were used in order to examine the relationship between such variables and the percentage of trained food selected. ANOVA analyses were also used to analyze total food eaten and jar climbs, which evaluated motivation to eat and explore, respectively. Additional mixed analyses of variance were carried out to analyze neophobia, with the dependent variables regular food (mean g of food eaten during the last rehabituation session prior to training) and new food (mean g of total food eaten, trained + untrained, during the test).

Regarding the olfactory test, an ANOVA analysis was applied considering group as the independent variable, and latency in finding the buried cookie as the dependent variable.

Experiment 2

Subjects

Electrophysiological assays were carried out in 10-week old male Wistar rats (Prolabor, Charles River Laboratories, Arbresle, France) that were housed under 12-h light/12-h dark cycle, in a temperature-controlled room (22ºC) with standard food and water available ad libitum, in accordance

with the European Communities Council Directive (86/609/EEC) for the care and use of laboratory animals.

Electrophysiological recordings in hippocampal slices

Baseline

Immediately after decapitation, animal brains were removed from the skull and dropped into a bubbled (95% O_2 and 5% CO_2) and ice-cold Krebs-Ringer bicarbonate (KRB) solution containing (nM):109 NaCl, 2.5 KCl, 1 KH_2PO_4, 1.3 $MgSO_4$, 2.5 CaCl2, 26.2 NaHCO3 and 11glucose. As described previously (Del Olmo et al., 2011), transverse hippocampal slices (400 μm) were cut with a manual tissue chopper (Stoelting Tissue Slicer, Stoelting, US) and placed in a humidified interface chamber at room temperature (20–25 °C). After a 2-h incubation period, slices were transferred to the submersion recording chamber at 31-32ºC which was continuously perfused with standard KRB solution at a rate of 1.8–2 ml/min. Field excitatory postsynaptic potentials (fEPSPs) were recorded for 100 min in hippocampal CA1 stratum radiatum with tungsten electrodes (1 MΩ) and evoked by stimulating Schaffer collateral–commissural fibers with biphasic electrical pulses (30–70 μA; 100 μs; 0.033 Hz) delivered through bipolar tungsten insulated microelectrodes (0.5 MΩ) every 15 s. Stimulus intensity was adjusted to evoke a response of 30%–40% of maximal fEPSP slope. The recording electrode was connected to an AI-402 amplifier (Axon Instruments, USA) plugged into a CyberAmp 380 signal conditioner (Axon Instruments). Electrical pulses were supplied by a pulse generator Master 8 (AMPI, Israel). Evoked responses were digitalized at 25–50 kHz using a Digidata 1322A (Axon Instruments) and stored on a PC compatible using pCLAMP 9.0 software (Axon Instruments). Drugs (DCS and SCOP, Sigma Aldrich, Spain) were prepared as stock solutions in PBS, stored frozen in the dark, and diluted to final concentration immediately before use. The synaptic strength was assessed by measuring the initial slope of the fEPSP. Data were normalized with respect to the mean values of the responses obtained by each animal at the 20 min baseline period. A single slice from each separate animal was considered as n=1.

Synaptic plasticity

In order to investigate the effects of the drugs, after obtaining stable synaptic responses for at least 20 min (baseline period), the different pharmacological treatments (DCS 50μM, DCS 100μM, SCOP 100μ, DCS 50μM + SCOP 100μM and DCS 100μM + SCOP 100μM) were perfused during 40 min. After 20 min of drug perfusion, Schaffer collateral fibers were tetanized with three 100-Hz pulses of 100 μs/sec duration every 20 s (high frequency stimulation, HFS) to induce saturated-LTP. Following LTP induction, recording was carried out during 60 min, 20 min with drug and 40 min with KRB solution alone. The synaptic strength was assessed by measuring the initial slope of the fEPSP, as analyzed with pCLAMP 9.0 software. The data were normalized with respect to the mean values of the responses obtained from each electrophysiological experiment during the 20 min baseline period.

Data analysis

To evaluate whether DCS administration in two different doses produced changes in the fEPSP responses, data were submitted to one-way ANOVA (PASW v20) in which the between-subject factor was group (VEH, DCS 50μM and DCS 100μM) and the within-subject factor was time before HFS. The time before HFS factor consisted of the average of the fEPSP slope in the last 20 min before LTP induction. In order to analyze the effects of the DCS in LTP, the averaged values of the initial slope of the fEPSP were analyzed by a repeated-measure ANOVA with the between-subject group factor (VEH, DCS 50μM, DCS 100μM) and the within-subject time factor (10 levels: the last 10 minutes of the recording). To evaluate the effect of DCS on LTP in SCOP-treated slices, a repeated-measure ANOVA was carried out, in which the between-subject factor was group (VEH, SCOP100μM, DCS 50μM + SCOP 100μM and DCS 100μM + SCOP 100μM) and the within-subject factor was time (the last 10 minutes of the recording). Finally, an additional ANOVA analysis was applied considering the group as the independent variable, which included 6 categories (VEH, DCS50μM, DCS100μM, SCOP100μM, DCS 50μM+SCOP 100μM and DCS 100μM+SCOP 100μM), and percentage of potentiation in the final recording (percentage of potentiation during the last 10 min of recording in relation to 10 min of the basal line prior HFS) as the dependent variable. Contrast analysis was performed by means of Fisher's LSD. Differences were considered significant when $p < 0.05$.

RESULTS

Experiment 1

Histology

At the end of the experiment, all observers were subjected to histological verification of correct bilateral cannula placements. For the final sample we only considered rats with their cannula tips bilaterally in the vHPC within the area delimited by CA3 and CA1, and the cannulae were located from -4.52 to -5.20 mm posterior to bregma (Fig. 1). Eight rats were excluded from behavioral data analyses since their cannulae were incorrectly implanted (n=5) or due to technical problems during the infusion (n=3). Thus, the final sample was made up of 42 subjects distributed into the following groups: VEH (n=10), DCS (n=10), SCOP (n=12) and DCS+SCOP (n=10).
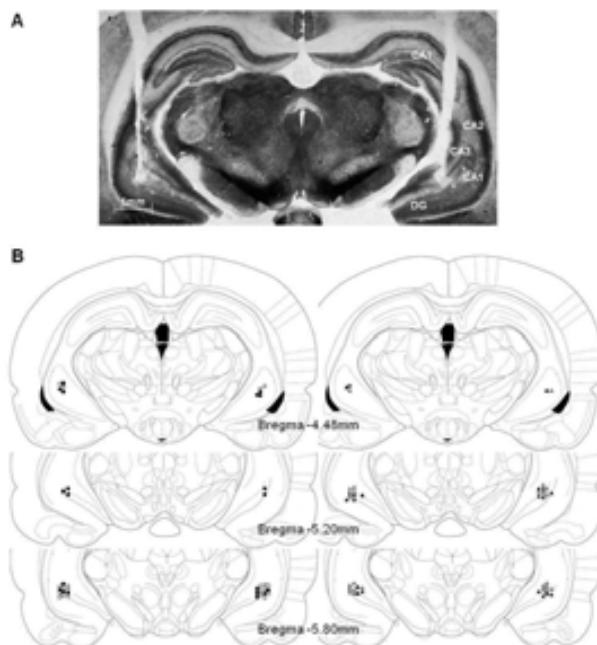
Figure 1. (A) Photomicrograph (10x) of acetylcolinesterase staining at the vHPC area (AP, 5.20 mm posterior to Bregma) showing the cannulae tracks and the microinjector tips of a representative subject. (B) Micro-injector tip placements throughout the rostral-caudal extent vHPC for each group: VEH (open circles), DCS (filled circles), SCOP (open triangles), and DCS+SCOP (filled triangles). [CA2: field CA2 of hippocampus, CA3: field CA3 of hippocampus, DG: dentate gyrus]

Behavioral testing

As depicted in Figure 2, statistically significant between-group differences in the percentage of trained food eaten were found [$F_{[3,41]}$=8.858, P=0.0001]. Contrast analyses showed statistically significant differences between SCOP and the remaining groups: VEH (P=0.006), DCS (P=0.002) and DCS+SCOP (P<0.0001). SCOP rats seem to exhibit no preference for the trained food since it was not significantly different from chance level ($t_{[11]}$=-1.675, P=0.122) whereas the other groups performed significantly above chance level ($t_{[9]}$'s > 3.41 and P's < 0.01). The analysis of the social interaction measures showed no statistically significant group effects in any of the variables (muzzle: $F_{[3,41]}$=1.880, P=0.149; body: $F_{[3,41]}$=1.522, P=0.224; anogenital: $F_{[3,41]}$=0.785, P=0.510; fighting: $F_{[3,41]}$=0.724, P=0.544 and grooming $F_{[3,41]}$=0.169, P=0.917). There were not statistically significant correlations either between such variables and the percentage of trained food (Table 1).

The analysis of the jar climbs performed in the test showed that the groups did not differ in investigation of both food jars ($F_{[3,41]}$=0.472, P=0.706). No statistically significant between-group differences were observed when the total amount of food consumed during the test session was analyzed ($F_{[3,41]}$=0.257, P=0.856). In the analysis of possible neophobic effects, mixed ANOVA showed a significant effect of food ($F_{[1,38]}$=4.611, P=0.038) but no significant effects of group ($F_{[3,38]}$=1.081, P=0.369) or group x food interaction ($F_{[3,38]}$=1.614, P=0.202) (Table 2).

The performance in this task did not seem to be related to changes in olfactory sensitivity since no statistically significant between-group differences were observed when the latency to find a buried sweet-smelling cookie was analyzed 24h after injections ($F_{[3,43]}$ = 0.139, P = 0.936).
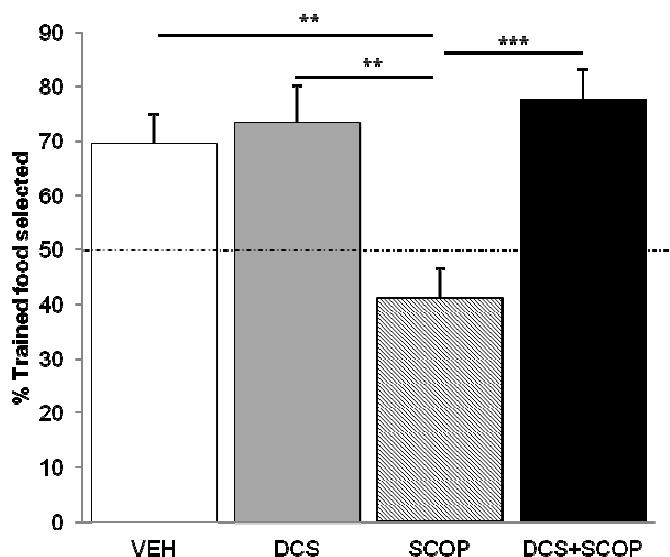
f the total amount of food compared to the remaining

Table 1. Variables measured in the acquisition session of the STFP task.

Group	Social Interaction				
	Muzzle	**Body**	**Anogenital**	**Fighting**	**Grooming**
VEH	24.8 ± 14.62	38.5 ± 19.3	23.5 ± 8.5	3.5 ± 2.01	3 ± 1.56
DCS	38.8 ± 16.23	44.2 ± 13.34	28.7 ± 9.42	3.6 ± 3.86	3.3 ± 2.58
SCOP	35.42 ± 15.37	45.58 ± 15.31	26 ± 11.51	4.42 ± 3.92	2.83 ± 2.08
DCS+SCOP	28.9 ± 12.02	32.5 ± 14.29	21.7 ± 13.4	2.3 ±3.14	3.12 ± 2.04
Pearson's Correlations	-0.11 (P=0.489)	-0.148 (P=0.351)	-0.118 (P=0.455)	-0.005 (P=0.973)	-0.132 (P=0.405)

Table 1. Means and ± SD of the number of sniffs (muzzle, body and anogenital zones), grooming and fighting scored during the acquisition session. Pearson's correlation coefficients (and P values) between such variables and percentage of food preference in the whole sample.

Table 2. Variables measured in the IV habituation and test session of the STFP task.

Group	Habituation	Test	
	Regular food	Total (new) food	Jar climbs
VEH	6.99 ± 2.28	6.99 ± 2.8	63.60 ± 13.90
DCS	8.86 ± 3.09	7.87 ± 3.59	56 ± 24.32
SCOP	8.19 ± 2.28	7.97 ± 2.38	49.8 ± 12.34
DCS+SCOP	10.49 ± 4.71	8.12 ± 3.92	64.6 ± 33.34

Table 2. Means ± SD of the amount of regular food eaten during the last rehabituation (unodorized ground food). Means and ± SD of the total amount of total odorized food eaten during the 24h STFP test (new food, trained + untrained). Means and ± SD of the number of jar climbs during the first 20 min of the STFP test.

Experiment 2

Effects of DCS on LTP (Fig. 3)

After a 20-min basal period and stable fEPSP responses, DCS 50μM (n=5) and 100μM (n=4) was added into the bath during 20 min in the corresponding groups. The ANOVA analysis of the fEPSP responses after 20 min of DCS perfusion (time before HFS), showed statistically significant between-group differences ($F_{[2,15]}$=6.098, P=0.014). The contrast analyses indicated that DCS (50 and 100μM) significantly increased the slope of synaptic responses (P=0.036 and P=0.006 respectively) compared with the fEPSP responses of the VEH group (n=7) during the basal period. Immediately after the HFS and following 20-min of DCS (50 or 100 μM) or VEH, the effects of the drugs on LTP (under 40 min of basal solution) were analyzed. The ANOVA analysis of the last 10 minutes of the recording (92-101 min, LTP maintenance) did not show statistically significant between-group differences ($F_{[2,13]}$=1.216, P=0.328), nor group x time interaction ($F_{[18,117]}$=1.132, P=0.331), but the time factor was statistically significant ($F_{[9,117]}$=2.205, P=0.026).
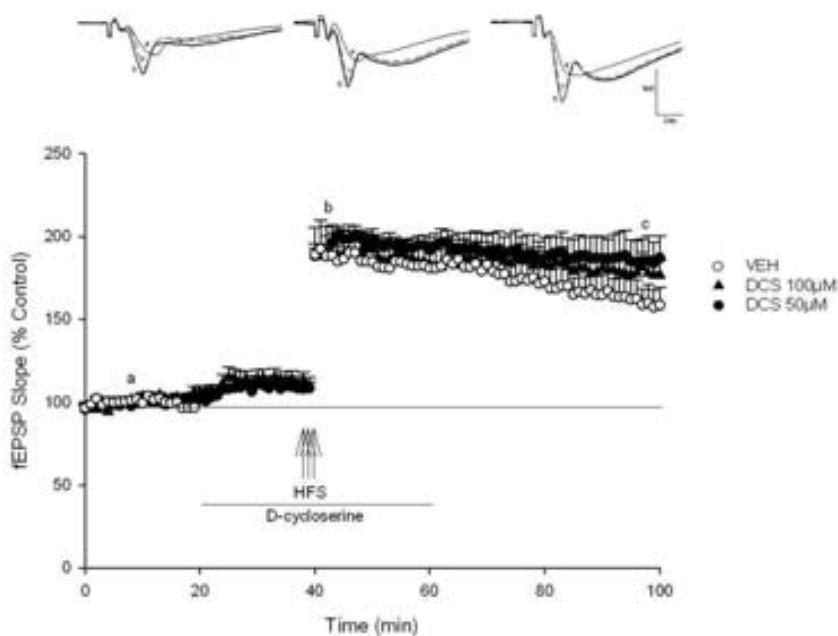
Figure 3. Effect of D-cycloserine on LTP. The symbols represent the fEPSP slope values from CA1 hippocampal slices in the VEH (open circles), DCS 50μM (filled circles) and DCS 100μM (filled triangles) groups. Slices from both DCS groups showed higher fEPSP slopes as compared to the VEH group. The upper traces are the averages of the fEPSPs recorded during the basal period (a, thin trace), after high frequency stimulation (HFS, indicated by arrows) (b, thick trace) and final record (c, dash trace). Calibration: 1 mV, 2 ms.

Effects of DCS and SCOP on LTP (Fig. 4 and Fig. 5)

After HFS and following 20 min of SCOP 100μM (n=8), DCS 50μM+SCOP 100μM (n=5), DCS 100μM+SCOP 100μM (n=4) or VEH (n=7), the effects of the drugs on LTP were analyzed. The ANOVA analysis of the last 10 minutes of recording showed that the group ($F_{[3,24]}$=3.204, P=0.041) and time ($F_{[4,96]}$=3.673, P=0.008) factors were statistically significant but not the interaction group x time [$F_{[12,96]}$=1.050, P=0.411]. Specifically, the group differences were found between the SCOP 100μM group and the remaining groups: VEH (P=0.016), DCS 50μM+SCOP 100μM (P=0.03) and DCS 100μM+SCOP 100μM (P=0.02). Additionally, the analysis of the percentage of potentiation in the final recording indicated statistically significant between-group differences [$F_{[5,31]}$=2.866, P=0.0306]. The differences were found between the SCOP 100μM group vs. VEH (P=0.019), DCS 50μM+SCOP 100μM (P=0.05), DCS 100μM+SCOP 100μM (P=0.05), DCS 50μM (P=0.002) and DCS 100μM (P=0.023).
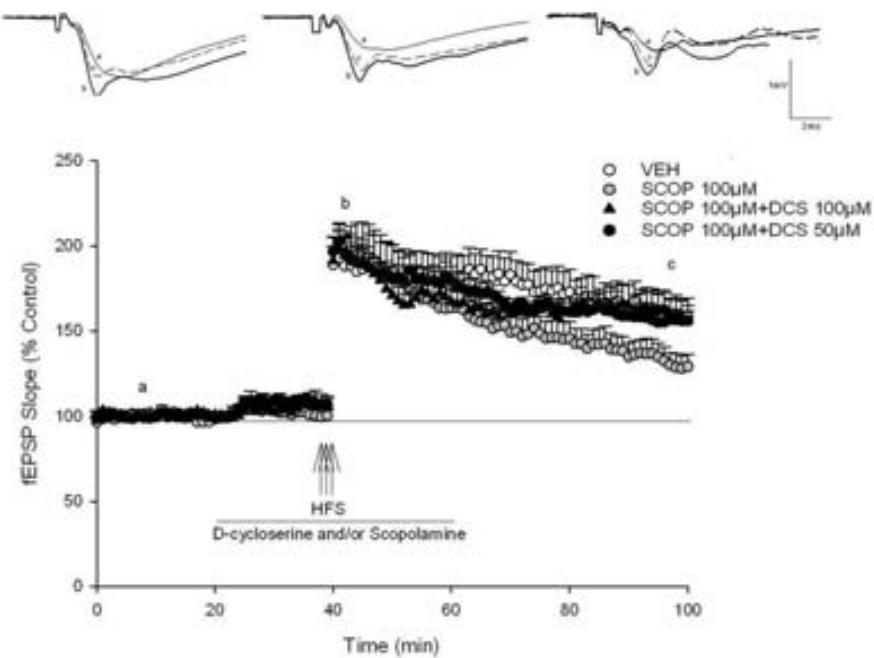
Figure 4. Effect of D-cycloserine and SCOP on LTP. The symbols represent the fEPSP slope values from CA1 hippocampal slices in the SCOP100μM + DCS 100μM (filled triangles), SCOP100μM + DCS 50μM (filled circles), SCOP 100μM (grey circle) and VEH (open circles) groups. Following the three trains of HFS, the SCOP100μM group showed a lower fEPSP slope as compared to VEH and both DCS treated groups. The upper traces are the averages of the fEPSPs recorded during the basal period (a, thin trace), after HFS (b, thick trace) and final record (c, dash trace). Calibration: 1 mV, 2 ms.
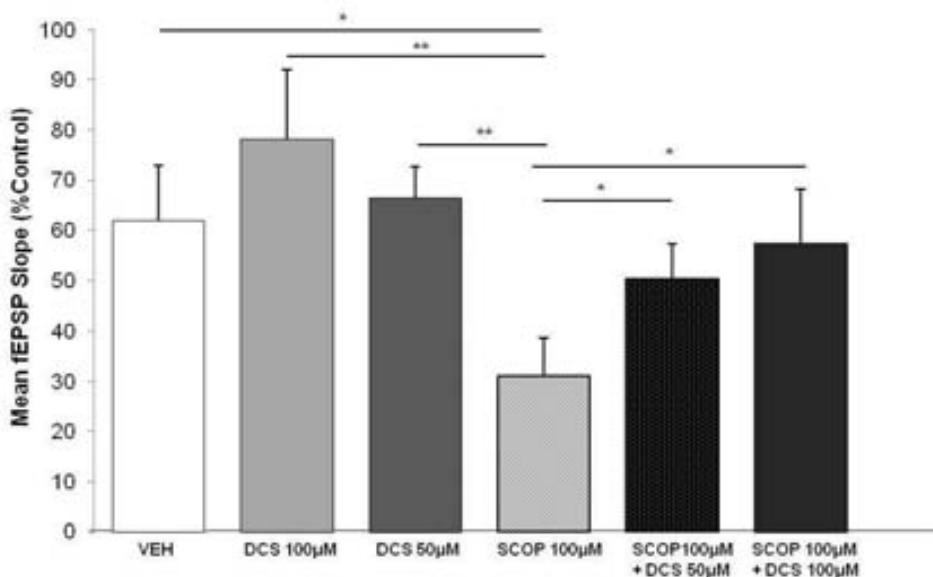
Figure 5. Percentatge of potentiation from the last 10 min of recording in all groups in control medium. The SCOP 100μM group significantly differed from the remaining groups (* $P<0.05$ and ** $P<0.01$).

DISCUSSION

In the present study, we provides evidence that potentiation of the glutamatergic activity in the hippocampus, with microinfusion of the partial NMDAR agonist DCS, rescues relational memory deficits in vitro LTP maintenance impairments induced by the administration of the muscarinic antagonist SCOP. Thus, vHPC administration of DCS prior to social interaction prevented the SCOP-

induced amnesia in the STFP memory test, as the trained food preference of the DCS+SCOP group significantly differed from the chance level and from that of the SCOP group but not VEH and DCS groups. Moreover, the analysis of the ancillary measures indicated that the effects of the infusions of DCS and/or SCOP in the STFP test cannot be attributed to alterations in olfactory perception, social investigation, neophobic responses or motor activity. The counteraction of SCOP-induced deficits found here is consistent with early reports suggesting that the hippocampus may be a critical region for the action of DCS in the reversion of SCOP-induced negative effects, although these effects had been demonstrated in spatial memory (Ohno and Watanabe, 1996; Kishi et al., 1998). Previous research also showed that systemic pre-learning injection of DCS attenuated SCOP-induced deficits both in spatial (Sirviö et al., 1992; Fishkin et al., 1993; Pitkänen, Sirviö, MacDonald, Ekonsalo, et al., 1995; Puumala et al., 1998) and non-spatial paradigms (Matsuoka and Aigner, 1996a; Andersen et al., 2002). Therefore, the present behavioral results add more evidence about a potential pervasive role of DCS in the reversion of memory deficits induced by different causes, such as cholinergic dysfunction, aging or brain lesions (for references see the Introduction section).

Moreover, the present results confirmed that the infusion of SCOP (40μg/hemisphere) in the vHPC immediately after the STFP acquisition produced a striking deficit in the 24h-memory test, observed by the poor performance of the SCOP group, which was not different from the chance level and significantly poorer than that of the VEH group. These data corroborate the contribution of muscarinic receptors in the consolidation of this naturalistic form of nonspatial relational memory since STFP impairments had also been observed when a lower dose of SCOP (20μg) was administered in the vHPC or the PLC (Boix-Trelis et al., 2007; Carballo-Marquez et al., 2009; Portero-Tresserra et al., 2013b). Likewise, the importance of muscarinic transmission in hippocampal-dependent learning and memory had already been reported in studies assessing spatial or contextual tasks. In this regard, SCOP administration in the vHPC disrupted the acquisition and consolidation of contextual fear conditioning (Wallenstein and Vago, 2001) and in the dorsal hippocampus impaired memory formation in radial (Mishima et al., 2000) and Morris water mazes (Herrera-Morales et al., 2007).

Regarding the lack of effect of DCS on memory in non-SCOP treated rats, this is consistent with data from previous experiments showing that DCS in the PLC ameliorated SCOP-induced deficits in the STFP task but did not enhance memory when injected in control rats, in contrast to what was found in a non-hippocampal dependent olfactory discrimination task (Portero-Tresserra et al., 2013b). Such findings suggest, as discussed in a previous study (Portero-Tresserra et al., 2013b), that DCS may have differential effects depending on the nature of the learning paradigm and may be interpreted as DCS enhancing implicit or procedural tasks, but its facilitative influence on relational hippocampal-dependent paradigms are more restricted. Indeed, the function of DCS on memory is still unclear since previous research that used systemic administration showed some inconsistencies in hippocampal-dependent paradigms (Lelong et al., 2001; Sunyer et al., 2008). Moreover, the memory process that is affected by DCS may also explain divergent results. Thus, in the present experiment we

analyzed the effects of the intra HPC DCS injected before learning but Ren et al., (2013), who found a beneficial impact of DCS, studied its effects on extinction.

As for the cellular mechanism underlying the reversion of SCOP-induced impairment, the current findings show, for the first time to our knowledge, those synaptic plasticity mechanisms may be involved. The results of the present study show that LTP maintenance in the hippocampus, which was markedly impaired after muscarinic blockade, can be restored with DCS, in agreement with the behavioral data demonstrating that STFP amnesia is rescued by DCS in the hippocampus. The present physiological results concur with the facilitative effect in hippocampal LTP demonstrated by GLYX-13, another NMDAR glycine-site agonist (Zhang et al., 2008), and also agrees with recent studies showing that DCS can reverse deficits in hippocampal LTP induced by head injury (Yaka et al., 2007), NCAM-deficiency (Kochlamazashvili et al., 2012) or aging (Billard and Rouaud, 2007). The SCOP-induced impairment in the maintenance of LTP was recovered with DCS in a dose-independent manner in SCOP treated-slices since the groups receiving 100 or 50 μM did not statistically differ in the percentage of potentiation. The current electrophysiological data also demonstrated that the SCOP (100 μM) perfusion in hippocampal slices significantly suppressed the LTP maintenance in CA1 synapses, as indicated by the lower percentage of potentiation in the last minutes of recording by the SCOP group vs. the remaining groups, confirming thus previous findings (Hirotsu et al., 1989; Ye et al., 2001; Lin et al., 2004; Sánchez et al., 2009). Moreover, similar detrimental effects of SCOP on LTP have been found in CA1 synapses in vivo(Ovsepian et al., 2004; Zhang et al., 2008) and also in other regions, e.g. corticostriatal synapses (Calabresi et al., 1999). In agreement with the above, it has been reported that muscarinic receptor activation facilitates the induction of LTP in rat hippocampal slices (Boddeke et al., 1992; Buchanan et al., 2010), which corroborates that the muscarinic receptors are a crucial event in this form of synaptic plasticity.

There are more lines of evidence suggesting such interaction between cholinergic and glutamatergic systems in mediating memory processes at the cellular level. It is known that the administration of ACh, acting on hippocampal muscarinic receptors, facilitates the "slow" component of EPSP mediated by NMDAr activation and increases the probability of generating NMDA-dependent LTP (Markram and Segal, 1990). In fact, cholinergic system activation must coincide with the release of glutamate to induce long-lasting plasticity (Navakkode and Korte, 2012). Moreover, the activation of muscarinic receptors can also lead to long-lasting depression of NMDAR-mediated synaptic transmission, via the induction of a form of synaptic plasticity (Jo et al., 2010). With regard to the specific muscarinic receptor subtype involved and the critical intracellular signaling pathways engaged, recent experiments have shown that M1 receptors enhanced NMDAR activation by inhibiting SK channels that otherwise act to hyperpolarize postsynaptic spines and inhibit NMDAR opening (Buchanan et al., 2010). Additionally, other mechanisms by which SCOP may disrupt LTP have been suggested, such as down-regulation of protein kinase C and nitric oxide (Saraf et al., 2010), which are required for LTP induction (Lu et al., 2000; Knafo et al., 2012) and maintenance (Muñoz et

al., 2000). Such interaction between NMDA and muscarininc systems is also observed in behavioral studies. For instance, concomitant administration of subthreshold doses of SCOP and NMDA antagonists systemically or into CA1 induced amnesia in inhibitory avoidance and spatial learning (Hlinák and Krejcí, 1998; Khakpai et al., 2012) and subbefective doses of NMDA and physostigmine exerted memory-enhancing properties (Jafari-Sabet, 2006).

Finally, the present experiment also indicated that the administration of DCS alone in CA1, prior to LTP induction, increased the magnitude of the fEPSP recorded. Thus, the groups DCS 50μM and DCS 100μM showed an increased percentage in the fEPSP slope from the 20-min baseline to the 20-min drug presence condition ($7.78 \pm 3.13\%$ and $11.85 \pm 4.37\%$ respectively) in contrast to VEH. Such results agree with previous studies (Rouaud and Billard, 2003) and concur with the importance of d-serine release in mediating synaptic plasticity (Henneberger et al., 2010). However, such between-group differences were not found after HFS, when LTP was induced, which indicates that the modulating effects of DCS on LTP may be due to methodological aspects. Although previous studies showed that the duration of the synaptic transmission potentiation was facilitated by DCS, they used a protocol inducing short-term potentiation (Rouaud and Billard, 2003). In the present experiment it has been used a protocol to induce a late-LTP and the three tetanus might produce a saturated potentiation.

Interestingly, in this study we found a correspondence between the results found at the behavioral and cellular levels. In both experiments, the administration of DCS was able to rescue SCOP-induced impairments since the DCS+SCOP groups showed comparable results in behavioral memory and electrophysiological recording. Moreover, the administration of SCOP produced memory deficits on both STFP retention and LTP maintenance. Additionally, DCS alone did not produce cognitive enhancing effects or improvements in the long-lasting potentiation. Consequently, DCS effects on STFP memory may be interpreted in terms of enhancing hippocampal LTP, considering this mechanism as a crucial element in memory or other forms of experience-dependent plasticity. Although there is not general agreement in considering LTP as a model for the synaptic changes that contribute to the neural circuit modifications underlying all types of experience-dependent plasticity, evidence continues to accumulate that LTP probably has important functional roles in many brain areas and under different circumstances (Malenka, 2003). Therefore, LTP may be one of the possible mechanisms that modulate the efficacy of synaptic transmission which are believed to stand for the physiological basis of learning mechanisms (Mayford et al., 2012).

Taken together; our results provide functional evidence that DCS may be helpful to reduce memory impairments associated with cholinergic depletion, probably by enhancing synaptic plasticity mechanisms. Hence, our findings support the use of NMDA-glycine site agonists, such as DCS, as potential treatments for alleviation of memory alteration occurring in neurodegenerative or neurologic diseases.

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4.   Experiment 7: Efectes de la DCS a l'HPC d'animals vells en la TSPA i el LAM

D-cycloserine reverses hippocampal-dependent memory deficits in aged rats

4.1 Theoretical approach

It has been described a potential involvement of cholinergic dysfunctions in age-related memory deficits since cholinergic neurons degenerate in the forebrain of Alzheimer's disease patients (Davies & Maloney 1976) and the administration of SCOP in adult rats disrupted learning in a similar way than in aged animals (Drachman & Leavitt 1974). Moreover, it has also been found a reduction in NMDA receptors with age that correlated with a decline in memory function, especially in hipocampal dependent tasks (Clayton et al., 2002), and with deficits in hippocampal synaptic plasticity (Stephens et al. 2011). In this context, some studies assessed the effects of DCS in aged rats, showing that systemic administration ameliorated deficits on hipocampal-dependent tasks such as Morris water maze (MWM) (Aura & Riekkinen 2000) and rescued hippocampal synaptic plasticity (Billard & Rouaud 2007). Nevertheless, although the hippocampus is currently being considered an early target of age-related structural and physiological changes, as far as we know, there are not previous experiments that studied intracerebral DCS to prevent aged memory problems.

In this context, the hypothesis of the present study was that the alteration of NMDAR transmission in the hippocampus is involved in the cognitive decline associated with age, especially in two hipocampal-dependent memory tasks such as MWM and STFP. In order to assess this hypothesis, we carried out this experiment:

- Experiment 7: The effects of Intra-vHPC DCS in the reversion of aged-related memory deficits in spatial and relational learning were assessed. DCS was administered 20 minutes before the acquisition of both tasks, and 72 hours later retention memory tests were conducted.
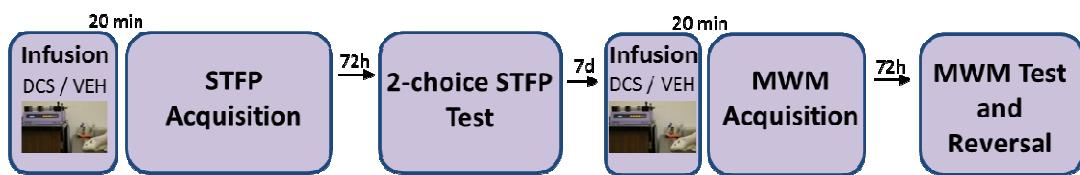
4.1.1 Methods and procedure (Figure 27)

The aim of this experiment was to analyze if the administration of intra-vHPC DCS (10µg/side) before STFP or MWT acquisition was able to facilitate memory deficits in aged rats. The dose of DCS chosen was the same than the one used in previous intracerebral studies (Lin et al. 2009).

To perform this experiment a final sample of 39 subjects in the STFP task (VEH-Old: n = 11, DCS-Old: n = 10, VEH-Young: n = 9, DCS-Young: n = 9) and 40 subjects in the MWM task (VEH-Old: n = 11, DCS-Old: n = 10, VEH-Young: n = 10, DCS-Young: n = 9) was considered. At the beginning of the experiment, the animals were habituated to the powered food and all underwent stereotaxic implantation of bilateral chronic guide cannulae into the vHPC. After the surgery, the rats were recovering for one week and then they were submitted to another habituation session and also adapted to a mock infusion protocol. Twenty minutes before STFP acquisition, the animals received a bilateral infusion of DCS (10µg) or VEH in a volume of 0.5µl/hemisphere for 2 minutes and they underwent the social interaction as explained in experiment 3. Seventy two hours after the STFP acquisition the retention test was performed and the preference score for trained food was calculated as a measure of memory retention. In this experiment, as it was performed in the previous ones, an olfactory perception test was conducted to rule out olfactory alterations due to the DCS infusions. Following one week, the animals started the training acquisition session in the MWM, which consisted of five consecutive days (four trials / day). In each trial, the animals learn to find the platform, guided by external cues considered spatial references and the latency to find the platform was scored. Twenty minutes before each acquisition session the animals received a bilateral infusion of DCS (10µg) or VEH as explained above. Seventy two hours after the last acquisition session the animals performed a drug free single-trial probe test, in which the platform was removed, and a reversal learning protocol, in which the platform was placed in the opposite quadrant than in the acquisition.
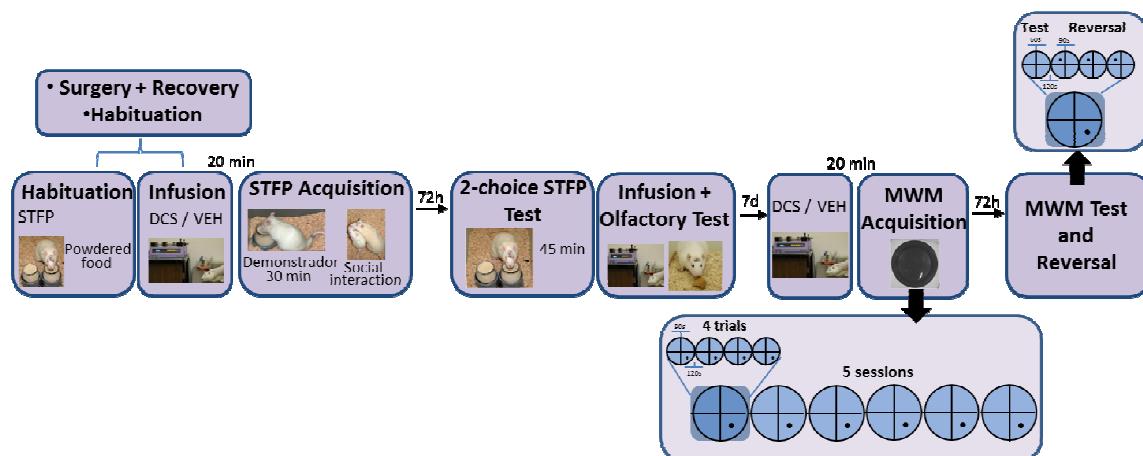
Figure 27. The behavioral procedure used for the experiment

4.1.2   Summary of the main results

- The physiological process of aging affected STFP memory since VEH-Old group exhibited a significantly lower percentage of preference for the trained food on the 72 hours retention test compared to VEH-Young.

- The potentiation of NMDAR in the vHPC due to DCS administration prior to the STFP acquisition enhanced memory in old animals. DCS-Old animals showed a higher food preference than VEH-Old group and their performance did not significantly differ from VEH-Young animals.

- The administration of DCS did not affect social interaction, neophobia, motor behavior or olfactory perception. However, the process of aging seems to produce a reduction in the social interaction during STFP acquisition, since Old rats performed fewer contacts than the Young rats. Aging also produced a reduction in olfactory detection in Old rats.

- The cognitive decline associated to aging affected MWM performance during the acquisition sessions and the reversal learning as VEH-Old rats showed higher latencies to find the platform than VEH-Young rats. Nonetheless, during the 72-hour probe test, all the rats significantly spent more time in the target quadrant, exhibiting correct memory retention.

- The administration of DCS intra-vHPC before MWM training did not enhance the old rats' performance during the acquisition or the probe test, but it seemed to improve cognitive flexibility since DCS-Old rats showed shorter latencies in finding the new location of the platform in the reversal trials performed after the probe test.

- During acquisition, the old animals did not differ on the path length and the thigmotactic behavior from the young animals. However, both old groups swam slower than young rats during acquisition and they showed a higher thigmotaxis during the test and the reversal learning.

- The infusion of DCS reduced the thigmotactic response on the test probe as DCS-treated animals showed less time swimming near the walls. However, this effect was not maintained in the reversal learning, in which DCS improved the new learning without affecting the thigmotactic behavior.

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ABSTRACT

It is well established that D-cycloserine (DCS), a partial agonist of the N-methyl-D-aspartate (NMDA) receptor glycine recognition site, may enhance learning and memory processes in diverse paradigms. Moreover, DCS has been shown to attenuate scopolamine-induced deficits in acquisition and memory consolidation of several tasks. Most studies have evaluated such role using systemic, intra-amygdala or prelimbic cortex DCS administration. The present project further investigates the brain areas where DCS may act on and explores whether DCS injected into the ventral hippocampus (vHPC) would reverse cognitive deficits found in old animals. For this purpose, we assessed the effects of pre-learning DCS (10 µg/hemisphere) in old and young rats in two learning tasks that depend on the hippocampus, the social transmission of food preference (STFP), which has no explicit contextual or spatial memory component, and the Morris water maze (MWM), which entails spatial information. The results showed that bilateral intra-hippocampal infusions of DCS rescued age-related deficits in STFP memory and MWM reversal learning, but did not enhance the MWM probe test. However, DCS did not improve young rats' memory in any of the two tasks. These results support DCS as a cognitive enhancer and corroborate that promoting NMDARs function in the vHPC may improve certain cognitive processes in aged animals. Present data may help in the search for strategies aimed at improving the alterations associated with the non-pathological process of aging.

1. Introduction

The physiological process of aging involves a decline of brain function that causes a progressive cognitive loss, which includes impairments in learning, memory (Yankner, Lu & Loerck, 2008) and other processes. Numerous studies indicate that the hippocampus, a critical region for spatial and non-spatial relational learning (Driscoll & Sutherland, 2005), is an early target of age-related structural and physiological changes that may contribute to impaired learning and memory, such as loss of synapses (Burke & Barnes, 2009), dysfunction in glutamate release (Stephens et al., 2011) and alterations in calcium homeostasis (Foster, 1999; Rosenzweig & Barnes, 2003). Another factor related to the cognitive impairment observed in aged animals is the reduced number of postsynaptic N-Methyl D-Aspartate receptors (NMDARs) and alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors (AMPARs) (Le Jeune et al., 1996; Rosenzweig & Barnes, 2003; Shi et al., 2007), which are essential for the expression of hippocampal synaptic plasticity involved in learning and memory (Martin, Grimwood & Morris, 2000). Moreover, an impaired

activation of the strychnine-insensitive glycine site of NMDARs by its endogenous agonist D-serine may also account for the deficits in hippocampal synaptic plasticity of memory-impaired aged rats (Potier et al., 2010). This suggests that activation of NMDARs at the glycine site may be an effective target for the development of treatments alleviating cognitive deficits in the elderly (Collingridge et al., 2013). Importantly, it also prevents the neurotoxicity that may result from activating the NMDA channel directly (Jansen & Dannhadrt, 2003).

One of the compounds exhibiting cognitive-enhancing properties (Monahan et al., 1989) that may be considered a promising candidate to improve learning and memory in aged animals is D-cycloserine (DCS), which acts as a partial agonist at the glycine-binding site of the NMDARs. It has been demonstrated in adult rodents that systemic administration of DCS rescued cognitive deficits due to sleep deprivation, pharmacological manipulations and brain injury (Andersen, Lindberg & Myhrer, 2002; Kranjac et al., 2013; Ohno & Watanabe, 1996; Silvestri & Root, 2008; Temple & Hamm, 1996; Waddell, Mallimo & Shors, 2010) and that intra-hippocampal administration enhanced spatial working memory deficits induced by blockade of glutamatergic or muscarinic receptors (Kishi, Ohno & Watanabe, 1998; Ohno & Watanabe, 1996). As for the effects of DCS on aged rats, systemic injections reversed learning and memory deficits on hippocampal-dependent memory paradigms such as Morris water maze (MWM) (Aura & Riekkinen, 2000; Aura, Riekkinen & Riekkinen, 1998; Baxter et al., 1994) and trace eyeblink conditioning (Thompson & Disterhoft, 1997). Such beneficial effects may be due to improvements in hippocampal synaptic plasticity mechanisms, suggested by a study showing that DCS increased the magnitude of synaptic potentials in hippocampal slices of both adult and aged rats (Billard & Rouaud, 2007). Moreover, a recent report indicated that DCS prevented the suppression of long-term potentiation induced by scopolamine in the hippocampus of adult rats (Portero-Tresserra et al., 2013).

Nonetheless, as far as we know, there are not previous studies evaluating the effects of DCS intracerebral administration in aged rats, which limits the knowledge of the specific brain areas in which this substance may act to improve age–related cognitive deficits. In this context, the aim of the present study was to examine whether DCS injected into the ventral hippocampus (vHPC), which seems more vulnerable than the dorsal hippocampus (dHPC) to the decrease in NMDARs and AMPARs associated with age (Liu, Smith & Darlington, 2008; Magnusson, Kresge & Supon, 2006), may ameliorate memory deficits in aged rats. For this purpose, two hippocampal-dependent learning paradigms were used, a task with no explicit contextual memory component, such as the social transmission of food preference (STFP) (Alvarez et al., 2001; Alvarez et al., 2002; Bunsey & Eichenbaum, 1995; Eichenbaum, 1999; Eichenbaum, 2000), and another involving spatial navigation, such as the MWM (Morris et al., 1982).

The STFP is an ethologically relevant test of olfactory memory in which an observer rat is allowed to interact with a demonstrator rat that has recently eaten a novel food (Galef & Whiskin,

2003). After such social interaction, observer rats significantly prefer the demonstrated food to a new food, although aged rats forget the preference more rapidly than young rats (Countryman & Gold, 2007). This task is sensitive to damage of several brain regions, including the hippocampus and related areas (Alvarez et al., 2002; Clark et al., 2002; Roberts & Shapiro, 2002; Ross & Eichenbaum, 2006; Winocur & Moscovitch, 1999), and increases in c-Fos expression and CREB phosphorylation have specifically been found in the vHPC following STFP acquisition and recall (Countryman et al., 2005; Smith et al., 2007). Additional experiments confirmed the relevance of this hippocampal region in the STFP, as injections of the muscarinic receptor antagonist scopolamine into the vHPC deteriorated memory (Carballo-Márquez et al., 2009), which was rescued by intra-vHPC administration of DCS in young rats (Portero-Tresserra et al., 2013).

The MWM (Morris, 1981) is one of the most widely used tests of spatial learning in studies of normal aging in rats (Kennard & Woodruff-Pak, 2011). In this task, rats learn and remember the location of an escape platform guided by a configuration of spatial cues surrounding the maze (Vorhees & Williams, 2006). It has been well established an age-associated decline in the MWM performance (Brandeis, Brandys & Yehuda, 1989; Gage, Dunnett & Björklund, 1984; Long et al., 2009; Van Der Staay & De Jonge, 1993; Yau et al., 2002), which has been related to physiological and structural changes in the hippocampus (Gallagher & Nicolle, 1993; Gallagher & Pelleymounter, 1988; Zhang et al., 2012). Although the dHPC is clearly involved in spatial learning and memory, including MWM (Fanselow & Dong, 2010; Moser & Moser, 1998; Levita & Muzzio, 2010), recent studies suggest that the vHPC may also play a role in spatial or contextual memory (Rudy & Matus-Amat, 2005; Ferbinteanu, Ray & McDonald, 2003; Loureiro et al., 2012), as place fields have been found in this hippocampal portion (Poucet et al., 1994; Kjelstrup et al., 2008; Fanselow & Dong, 2010; Jung et al., 1994).

Therefore, in the present study we analysed the effects of DCS infusions (10 μg / hemisphere) into the vHPC in 23-month old rats. In the STFP, the DCS treatment was administered 20 min before acquisition (social interaction) and memory was assessed in a subsequent drug-free 72-h test. In the MWM, DCS was administered 20 min before each of the five acquisition sessions and memory was evaluated in two drug-free sessions, a 72-h probe test and a reversal learning test. The enhancement of the glutamatergic transmission in the hippocampus might positively modulate the cognitive decline due to aging. In order to investigate this hypothesis, we have designed an experiment aimed to evaluate the effects of DCS infusion into the vHPC in old rats with two hippocampal-dependent tasks, one of them entails spatial information and the other one involves the olfactory system.

2. Materials and methods

2.1. Subjects

Forty-six male Wistar rats, experimentally naïve, belonging to our laboratory's breeding stock were used. At the beginning of the experiment, 25 were old (mean age = 23.61 mo; mean weight = 499.88 g, SD = 66.48) and 21 young (mean age = 3.1 mo; mean weight = 371.7 g, SD = 26.49). The old rats followed a food restriction schedule since they were four months old (18 - 20 g / d) in order to diminish the risk of cardiovascular diseases and reduce the mortality rate (Heilbronn & Ravussin, 2003). An additional set of 28 juvenile male Wistar rats (mean age = 1.9 mo, mean weight = 253.17 g, SD = 27.71 at the beginning of the experiment) served as demonstrator subjects in the STFP task. All the rats were single-housed in 50 x 22 x 14-cm plastic-bottomed, sawdust-bedded cages in a room controlled for temperature (20 - 22 ºC) and humidity (40% - 70%), with the exception of the juvenile rats, which were pair-housed. The rats were maintained on an artificial 12 h light-dark cycle (lights on at 8:00 a.m.), with experiments performed during the first half of the light cycle. Rat-chow pellets (Scientific Animal Food & Engineering, Augy, France) were provided *ad libitum* to young rats except during habituation, acquisition and test sessions of the STFP task, in which the young and old rats were submitted to a food restriction schedule (12 g / d to maintain body weight at 85% of their initial weight). The animals were handled on a daily basis for 5 min. All procedures were carried out in compliance with the European Community Council Directive for care and use of laboratory animals (86 / 609 / ECC) and with the Generalitat de Catalunya's authorization (DOGC 2450 7 / 8 / 1997, DARP protocol number 5959). All efforts were made to minimize the number of animals used and their suffering.

2.2. Surgery

Animals were i.p. anesthetized with 150 mg/kg Imalgène ketamine chlorhydrate (Merial, Lyon, France) and 0.08 mg/kg Rompun xylazine (Bayer, Barcelona, Spain) and placed in a stereotaxic head holder (Stoelting, ST51904 Digital Manipulators Arm, Illinois, EEUU). The skull was exposed through a mid-line incision and leveled along the bregma-lambda axis. All experimental animals, except juvenile rats, underwent stereotaxic implantation of bilateral chronic guide cannulae into the vHPC. Each guide cannula comprised one 26-gauge metal tube projecting 7.5 mm from the pedestal (Plastics One, Bilaney Consultants GMBH, Düsseldorf, Germany). The stereotaxic coordinates for implantation in the vHPC were: anteroposterior: -5 mm from bregma; mediolateral: ±5 mm from midline; and dorsoventral: -6.8 mm from cranium surface (Paxinos & Watson, 1997). Sterile dummy stylets (Plastics One) were placed into the cannulae to prevent occlusion. After surgery, the rats were administered an antibiotic (Panolog, Novartis, Huninge, France) and were returned to their home cages for 10 days (5 for recovery, 4 for food restriction and 1 for rehabituation) before behavioral training.

During the 10-day recovery period, the rats were handled and weighed on a daily basis and the dummy stylets were changed every other day.

2.3. Microinfusion procedure

One day prior to STFP and MWM training, all the rats, except the juvenile, were adapted to a mock infusion protocol (no solutions injected) in order to minimize any stress associated with the procedure. The following day, the rats received the drug infusions (DCS or vehicle) 20 min before the acquisition sessions. For this purpose, they were gently restrained while the dummy stylets were removed and replaced with two 33-gauge stainless-steel injectors (Plastics One) extending 1 mm below the cannulae tips. The injectors were connected by polyethylene tubing (Plastics One) to two 10 μl syringes (SGE Analytical Science, Cromlab S.L., Barcelona, Spain) mounted in an infusion pump (11 Plus Syringe Pump, Harvard Apparatus Inc., Holliston, Massachusetts, USA). DCS (Sigma-Aldrich, Madrid, Spain) was dissolved in phosphate-buffered saline (PBS, 0.1 M, pH 7.4) and a dose of 10 μg / hemisphere was administered into the vHPC. The rats in the VEH groups received PBS injections. The solutions were infused bilaterally in a volume of 0.5 μl / hemisphere for 2 min. The injectors were left in place for 1 min after the infusion was complete to allow for diffusion. Such injection parameters were selected on the basis of previous studies in which 10 μg / hemisphere of DCS in the vHPC reversed deficits produced by scopolamine in STFP memory consolidation (Portero-Tresserra et al., 2013). The same dose of DCS also produced beneficial effects on memory when injected in other brain regions such as the basolateral amygdala (BLA) or the prelimbic cortex (PLC) (Portero-Tresserra et al., 2013; Villarejo-Rodríguez et al., 2010).

2.4. Apparatus

In the STFP task, all observers were habituated, trained and tested in their own 50 x 22 x 14-cm plastic-bottomed, sawdust-bedded cages. Habituation and testing were carried out using a feeding-tray placed in the animals' cages. The tray consisted of a black Plexiglas base (21 x 21 cm) with two adjacent plastic pots fixed onto the center of the base. The food (powdered rat chow) was placed in 130-ml glass jars secured within each plastic pot. For the demonstrators, habituation to eat powdered food was carried out in 50 x 22 x 14-cm plastic cages in which they were allowed to eat from a glass jar mounted upon the center of a black Plexiglas base (21 x 10 cm). For the acquisition and test, powdered rat chow was 2.2% ground cocoa (Oxfam Fairtrade, Gent, Belgium) and 1% ground cinnamon (Carmencita, Alicante, Spain), based on previous studies in which the subjects did not show innate flavor preference (Boix-Trelis et al., 2007; Wrenn et al., 2003). All sessions were recorded by a video camera (JVC, Everio Model GZ-X900) connected to a monitor.

The MWM consisted of an elevated circular pool with the walls painted in black (2 m diameter; 60 cm above the floor) which was filled to a height of 45 cm with water maintained at 22 ± 2 °C. The pool was placed in the middle of a dark room and surrounded by black curtains forming a

circular enclosure (2.4 m in diameter). Animals were required to escape from the water onto a small 11-cm platform hidden just 2 cm beneath the water surface. The location of the platform could only be encoded relative to distal visual landmarks surrounding the MWM. Thus, different stable visual cues were placed inside the enclosure: a plastic beach ball with alternate blue, white, yellow, white, orange and white vertical segments, a white box with horizontal black stripes, a brown teddy bear, an illuminated region of the black curtain and a white box with a light inside. In all the sessions, the swim paths of the animals were recorded using a video camera connected to a computer running a tracking-software (Smart Video Tracking System, Version 2.5, Panlab, Barcelona, Spain), which calculated the analyzed parameters (see Results).

2.5. Behavioral procedure

All the animals performed first the STFP task, then an olfactory perception test and, finally, the MWM task. Subjects were administered the same substance (PBS or DCS) across all behavioral procedures.

2.5.1. Social transmission of food preferences

2.5.1.1. Habituation

After 5 days of food restriction, prior to surgery, observers and demonstrators were habituated to powdered chow (Scientific Animal Food & Engineering, Augy, France) from glass jars to minimize neophobia. The observers were habituated for 2 h on the first day, 1 h the second day and 45 min the third day, and the demonstrators were habituated for 45 min the 3 days. A similar procedure was repeated 9 days after surgery for the observers (one 45-min rehabituation session), where the variable *Regular ground food* (mean g of food eaten) was recorded. Subsequently, animals were food-restricted once again for 1 day before the training–testing sessions began.

2.5.1.2. Acquisition and Test

Before training was conducted, each observer rat was assigned randomly to be cued to one flavor (cocoa or cinnamon), which was counterbalanced in each group. The task began when a demonstrator rat was allowed to eat food flavored with cocoa or cinnamon for 30 min. During the 30-min period, the observers received a bilateral intra-vHPC infusion of PBS or DCS and then they were returned to their cages. Twenty min after the infusion, the acquisition session took place. A demonstrator that had just eaten flavored chow was placed into the observer's cage and the two rats were allowed to interact with no barriers for 30 min, following which the demonstrator was removed. In the acquisition, the number of times each observer sniffed the muzzle, body or anogenital region of the demonstrator was scored, as well as grooming and fighting bouts, during the 15 first minutes of the interaction. A sniff was defined as close orientation (< 2 cm) of the observer's muzzle toward the demonstrator (Wrenn et al., 2003).

The STFP test was performed 72-h after acquisition (Countryman & Gold, 2007) and consisted of placing two jars filled with flavored food and with water available. One of the jars contained the chow with the flavor that was given to demonstrators (trained food) and the other jar contained a different scented chow (untrained food). The observers were allowed to eat for 45 min, after which the food jars were removed and weighed to determine the amount of food eaten from each, and only rats eating at least 2 g of food were included in the study (all rats met this criterion). A preference score for the trained flavor (*Percentage of trained food*) was calculated as follows: 100 x (weight of trained food eaten / weight of all food eaten). In the test, the *Flavored food* eaten (mean g of total food eaten, trained + untrained) and the *Jar climbs* (number of times the observer was on top of the jar with both forepaws during the first 15 min of the test) were also scored.

2.5.2. Olfactory perception test

To rule out olfactory alterations due to the DCS infusions, an olfactory perception test was conducted at the end of the experiment (Wrenn et al., 2003) on a sample of subjects (n = 7 for each group). Twenty-four hours before the olfactory test, the rats were habituated to butter-flavored cookies (Brambly Hedge, Denmark) and then they were food-restricted until the test took place. The following day the rats were infused with DCS or PBS and, 20 min later, the test was conducted in clean rat cages (50 x 22 x 14-cm) where a piece of cookie was buried in one of the corners. The rats were then placed in the cage, and the *Latency* to find the buried cookie and commence eating was timed.

2.5.3. Morris water maze

2.5.3.1. Acquisition

The training acquisition sessions in the MWM consisted of four daily trials (average intertrial interval, ITI, 120 s) for five consecutive days. In each trial, the rats were placed facing the edge of the pool, in a new starting position for each trial (randomly N, S, E or W), and they were required to find the platform whose position remained stable across trials and days in the SE quadrant. If the animal failed to find the platform within 90 s it was manually guided to the platform and after 15 s it was removed from the pool. When a rat found the platform it was left on it for 15 s and then removed from the pool. In the acquisition, the *Latency* to find the hidden platform (time needed to find and climb onto the platform), *Thigmotaxis* (percentage of time spent near the walls), *Length* (total distance swum) and *Speed* (total distance / time) were scored.

2.5.3.2. Probe test and reversal learning

Seventy-two h after the last acquisition session, the platform was removed from the pool and each rat was given a drug-free single-trial probe test. Starting from the E cardinal point, the rats were allowed to freely swim for 60 s. The variables analyzed were *Percentage of time spent in the target quadrant* (time spent in the target quadrant versus the time spent in the remaining quadrants), *Distance swum in the target quadrant* (total distance swum within the target quadrant), *Distance to target* (the

average distance of the rat during swimming to the spatial location at which escape platform was located during training) and the control variables *Thigmotaxis*, *Length* and *Speed*. Successful spatial learning was indicated by a clear preference for the goal quadrant and by decreases in the total length that the rat swam in order to locate the platform and escape (Gallagher, Burwell & Burchinal, 1993).

Once the test was over, a drug-free reversal learning protocol was included in which the platform was placed in the opposite quadrant (NW) than in the acquisition. First, the experimenter guided the rat to the platform and after 15 s in it, the rat was removed from the pool. Then, it was administered a set of three trials (average ITI 120 s), keeping the platform in the NW quadrant. During reversal learning, *Latency* in finding the hidden platform, *Percentage of time in the SE quadrant*, *Percentage of time in the SE target annulus* and the same control variables as in the acquisition and test were studied. Reversal learning evaluates if the rats can inhibit the initial learning of the platform location and acquire a new learning, which implies a flexible cognitive map (Vorhees & Williams, 2006).

2.6. Histology

Upon completion of the behavioral testing, a sample (VEH-Old: n = 5, DCS-Old: n = 5, VEH-Young: n = 6, DCS-Young: n = 6) of the subjects was perfused (the remaining of the sample was beheaded for further analyses not shown here). The rats were deeply anesthetized with an overdose of sodium pentobarbital (Dolethal, Vetoquinol S.A. Madrid, Spain; 200 mg / kg intraperitoneal) and perfused transcardially with PBS followed by 4% paraformaldehyde in 0.1 M PBS at a flow rate of 40 ml / min. The cannulae were carefully removed and brains were postfixed in paraformaldehyde for 2 h and then submerged in a 20% sucrose solution prior to sectioning. Coronal 40-µm sections were cut on a cryostat (Shandom Cryotome FSE, Thermo Electron Corporation, Waltham, Massachusetts, USA), mounted and processed for acetylcholinesterase histochemistry, essentially as described elsewhere (Paxinos & Watson, 1997). The sections were examined under a light microscope (Olympus BX 41; Olympus Optical CO, LTD, Tokyo, Japan) and microphotographs of the cannulae placements were taken using a digital camera (Olympus DP70).

2.7. Data analysis

All statistical analyses were carried out using SPSS software (SPSS v18 Science, Chicago, IL, USA). First, in order to examine the evolution of the subjects' body weight over the course of the experiment, data were submitted to a mixed ANOVA in which the between-subject factors were *Age* (Old or Young) and *Drug* (VEH or DCS) and the within-subject factor was S*ession,* including 16 measures of different representative days of the experiment. The analyses of the variables in the STFP and MWM were performed by means of ANOVA with *Age* (Old or Young) and *Drug* (VEH or DCS) factors as the independent variables. P-values less than 0.05 were considered to be significant.

Regarding the STFP task, ANOVA analyses were used to analyze the *Percentage of trained food* (as a measure of memory retention), the *Total food eaten* (measuring motivation to eat) and *Jar climbs* (evaluating motor behavior and motivation to explore). In addition, a one-sample t test against a constant (50) was used for each group to determine whether the *Percentage of trained food* eaten was different from the chance level (50%). Further mixed ANOVA analyses were carried out to analyze neophobia, with the dependent variables *Regular ground food* and *Flavored food*. To evaluate whether all the animals had similar opportunities of learning (similar social interaction levels), we carried out another mixed ANOVA analysis, considering *Sniffs of the demonstrator's muzzle*, S*niffs of the demonstrator's body*, S*niffs of the demonstrator's anogenital region*, *Self-grooming bouts* and *Fighting bouts* as the dependent variables. Pearson's correlation tests were used to examine the relationship between such variables and the *Percentage of trained food*. Regarding the olfactory test, an additional ANOVA analysis was applied considering *Latency* in finding the buried cookie as the dependent variable.

In the acquisition of the MWM, data were submitted to a mixed ANOVA with *Session* as a within-subject factor that consisted of 5 measures (Day 1 - Day 5, the average scores of the four trials for each day). ANOVA analyses were used to study the *Latency* in finding the hidden platform and the control variables *Thigmotaxis*, *Length* and *Speed*. In the probe test, ANOVA analyses were conducted in order to examine the *Percentage of time spent in the target quadrant*, the *Distance swum in the target quadrant*, the *Distance to target* and the control variables mentioned above. In addition, a one-sample t test against a constant (25) was used for each group in order to evaluate whether the *Percentage of time spent in the target quadrant* was different from the chance level (25%) analyzing the whole trial (60 s) and two periods of it (the first 30 s and the second 30 s). Concerning the reversal learning, the data analyzed were the average score of the three trials in the following variables: the *Latency* in finding the hidden platform, the *Percentage of time spent in the SE quadrant*, the *Percentage of time spent in the SE target annulus* and the same control variables as in the acquisition and test.

3. Results

3.1. Histology

For a correct cannulae implantation, the injector tip had to be located bilaterally within the vHPC, within the area delimited by CA3 and CA1 and in which no tissue damage resulting from the rate or volume of the infusions was detected (Fig. 1A). Specifically, the subjects included in the final sample had the cannulae located along different brain coordinates from 4.52 to 6.04 mm posterior to bregma (Fig. 1B) according to the stereotaxic atlas (Paxinos & Watson, 1997). Eight rats were excluded from behavioral data analyses due to technical problems durign the drug infusion (n=2), their

cannulae were unintentionally implanted outside vHPC (n=4) or they were statistically descrived as a outliers during the behavoioral analyses (n=2). Therefore, the final sample after application of exclusion criteria included 39 subjects in the STFP task (VEH-Old: n = 11, DCS-Old: n = 10, VEH-Young: n = 9, DCS-Young: n = 9) and 40 subjects in the MWM task (VEH-Old: n = 11, DCS-Old: n = 10, VEH-Young: n = 10, DCS-Young: n = 9).

3.2. Behavior

3.2.1. Social transmission of food preference and olfactory perception test

The ANOVA analysis revealed no statistically significant effects of *Age* ($F_{[1,35]}$ = 1.519, P = 0.226) or *Drug* ($F_{[1,35]}$ = 2.285, P = 0.140), but there was a statistically significant *Age x Drug* interaction ($F_{[1,35]}$ = 6.291, P = 0.017) in *Percentage of trained food eaten* in the test (Fig. 2). The contrast analyses revealed statistically significant differences between the VEH-Old group and the DCS-Old (P = 0.006) and VEH-Young groups (P = 0.011). The t-test analysis showed that the VEH-Old group exhibited a preference for the trained food that was not statistically different to the chance level ($t_{(10)}$ = 1.307, P = 0.22). In contrast, the DCS-Old ($t_{(9)}$ = 8.995, P = 0.000), VEH-Young ($t_{(8)}$ = 5.312, P = 0.001) and DCS-Young groups ($t_{(8)}$ = 3.441, P = 0.009) performed significantly above the chance level, demonstrating STFP retention.
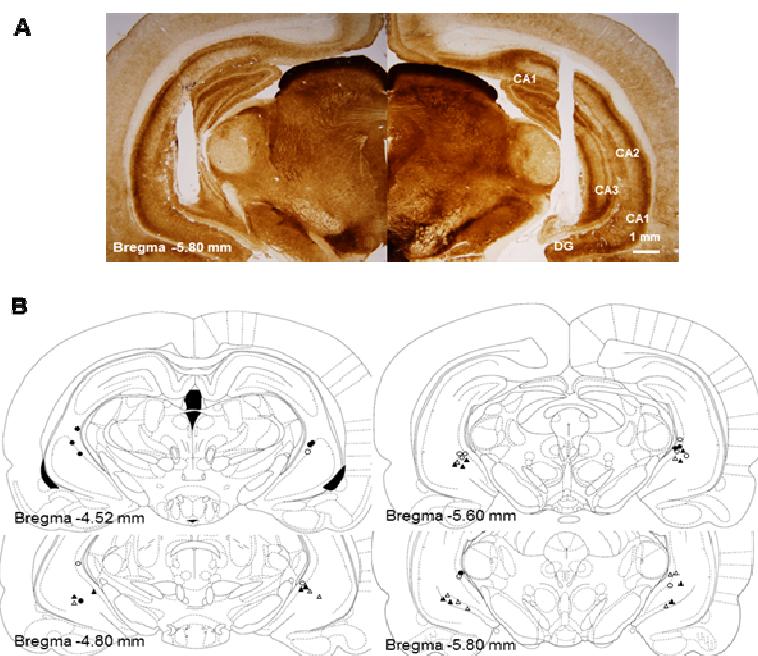
Figure 1. (A) Photomicrograph (magnification x10) of acetylcholinesterase histochemistry at the level of the ventral hippocampus showing the cannula tracks of a representative subject. (B) Cannula tip placements (microinfusors extending 1mm below) for VEH-Old (white dots), DCS-Old (black dots), VEH-Young (white triangles) and DCS-Young (black triangles) groups.
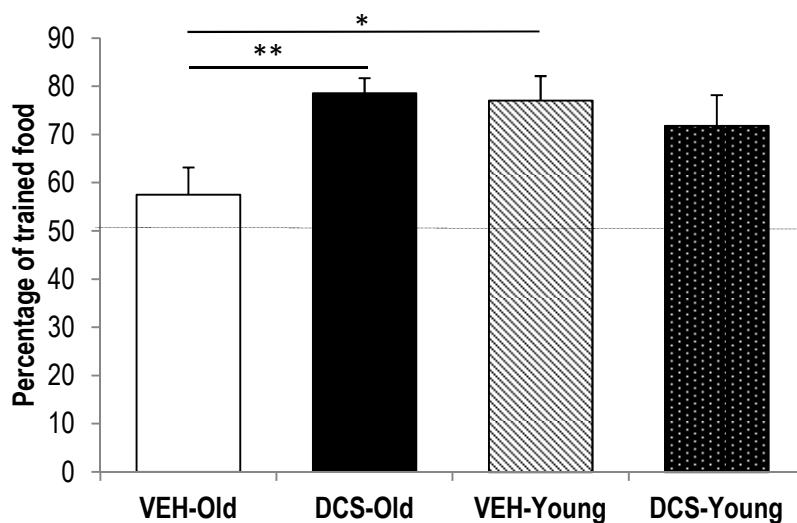
Figure 2. Percentage of trained food eaten (±SE) in the STFP test for each group. * P < 0.05 ** P < 0.01

The analysis of the variables of the social interaction (Fig. 3) revealed statistically significant *Age* effects in *Sniffs of the demonstrator's muzzle* ($F_{[1,35]}$ = 4.407, P = 0.042), S*niffs of the demonstrator's body* ($F_{[1,35]}$ = 12.834, P = 0.001) and S*niffs of the demonstrator's anogenital region* ($F_{[1,35]}$ = 27.998, P < 0.0001), while there were no significant *Age* effects in the variables *Self-grooming bouts* ($F_{[1,35]}$ = 0.602, P = 0.443) and *Fighting bouts* ($F_{[1,35]}$ = 3.578, P = 0.067). In addition, in any of the variables, there were no *Drug* significant effects (*Sniffs of the demonstrator's muzzle*: $F_{[1,35]}$ = 0.648, P = 0.426; S*niffs of the demonstrator's body*: $F_{[1,35]}$ = 1.359, P = 0.252; S*niffs of the demonstrator's anogenital region*: $F_{[1,35]}$ = 0.095, P = 0.760; *Self-grooming bouts*: $F_{[1,35]}$ = 0.082, P = 0.776; *Fighting bouts*: $F_{[1,35]}$ = 0.526, P = 0.473) neither *Age x Drug* interaction (*Sniffs of the demonstrator's muzzle:* $F_{[1,35]}$ = 0.011, P = 0.917; S*niffs of the demonstrator's body*: $F_{[1,35]}$ = 0.065, P = 0.801; S*niffs of the demonstrator's anogenital region*: $F_{[1,35]}$ = 0.003, P = 0.956; *Self-grooming bouts*: $F_{[1,35]}$ = 0.082, P = 0.776; *Fighting bouts*: $F_{[1,35]}$ = 1.761, P = 0.193). In addition, there were no statistically significant correlations between such variables and the *Percentage of trained food* (Muzzle: r = -0.201, P = 0.219; Body: r = -0.099, P = 0.547; Anogenital region: r = 0.132; P = 0.423; Self-grooming: r = 0.304, P = 0.06; Fighting: r = 0.017, P = 0.92).
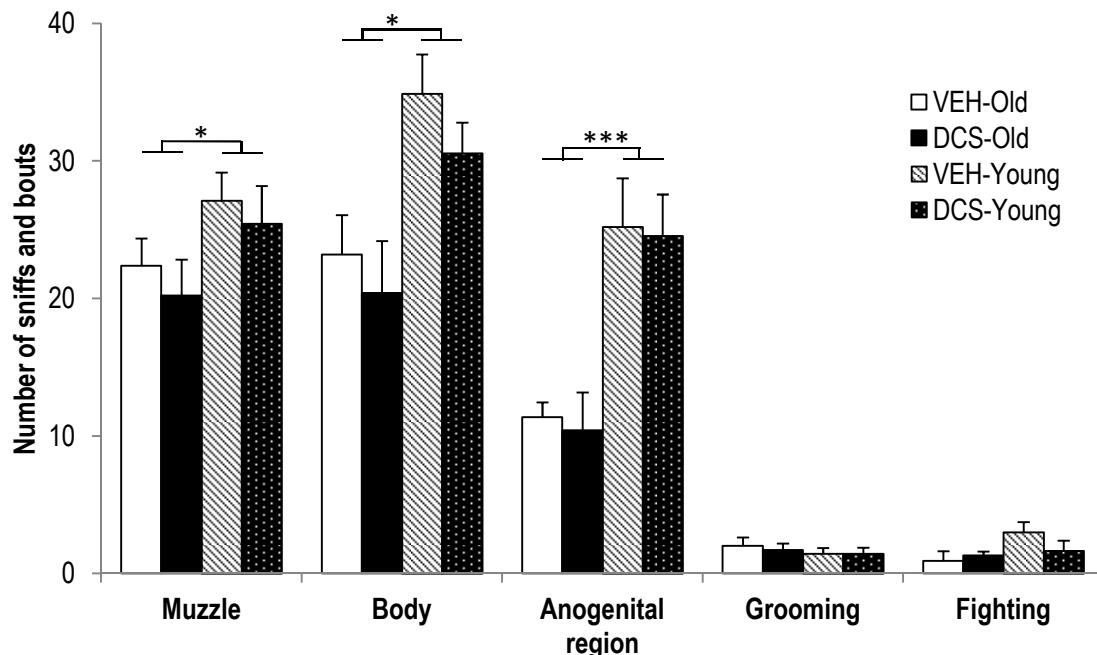
Figure 3. Number of sniffs (±SE) of the demonstrator's muzzle, body and anogenital region and self-grooming and fighting bouts for each group during the social interaction in the STFP acquisition. * P < 0.05 *** P < 0.001

In the analysis of neophobia, a mixed ANOVA analysis showed no statistically significant effect of *Food* ($F_{[1,35]}$ = 3.245, P = 0.08), *Food x Drug* ($F_{[1,35]}$ = 0.563, P = 0.458) or *Food x Age x Drug* interaction ($F_{[1,35]}$ = 0.014, P = 0.905), suggesting that DCS did not produce changes in neophobia (Table 1). However, there was a statistically significant *Food x Age* interaction ($F_{[1,35]}$ = 7.366, P = 0.010), indicating that during the rehabituation session, the Old rats ate less *Regular ground food* compared with the young rats. Regarding food consumption during the test, the statistical analyses did not demonstrate statistically significant effects of *Age* ($F_{[1,35]}$ = 0.272, P = 0.605), *Drug* ($F_{[1,35]}$ = 0.113, P = 0.739) or *Age x Drug* interaction ($F_{[1,35]}$ = 0.468, P = 0.498) in the *Total food eaten* during the test, showing that DCS did not affect motivation to eat (see Table 1).

Group	Rehabituation session		Test	
	Mean	Std. Deviation	Mean	Std. Deviation
VEH-Old	6.173	±5.738	9.2	±6.153
DCS-Old	7.08	±5.471	11.09	±7.022
VEH-Young	12.822	±6.447	11.433	±3.908
DCS-Young	10.822	±4.771	10.789	±5.224
Total	9.013	±6.069	10.567	±5.604

Table 1. Comparison between the consumption (g ±SD) of regular ground food (rehabituation session) and flavored food (test) for each group.

Regarding motor behavior and motivation to explore, data from the test session indicated no statistically significant *Age* ($F_{[1,35]}$ = 0.72, P = 0.402), *Drug* ($F_{[1,35]}$ = 0.579, P = 0.452) or *Age x Drug*

interaction ($F_{[1,35]}$ = 0.012, P = 0.912) in the variable *Jar climbs*, showing that all the rats climbed onto the jars in a comparable way.

Finally, performance was not related to deficits in olfactory sensitivity due to DCS administration since no statistically significant *Drug* effects ($F_{[1,24]}$ = 1.125, P = 0.299) or *Age x Drug* interaction ($F_{[1,24]}$ = 0.057, P = 0.813) were observed when the variable *Latency* in finding the buried cookie was analyzed. Nevertheless, the factor *Age* was statistically significant ($F_{[1,24]}$ = 4.556, P = 0.043), confirming that the old rats spent more time in finding the buried cookie.

3.2.2. Morris water maze

In order to assess the learning process during acquisition, the *Latency in finding the hidden platform* was evaluated (Fig. 4). An ANOVA analysis showed statistically significant effects of *Session* ($F_{[4,144]}$ = 19.677, P < 0.0001) and *Age* ($F_{[1,36]}$ = 17.923, P < 0.0001), but there were no effects of D*rug* ($F_{[1,36]}$ = 1.779, P = 0.191) or interaction *Age x Drug* ($F_{[1,36]}$ = 0.2, P = 0.657). The contrast analyses showed statistically significant differences between old rats and young rats (P < 0.0001) when considered all the acquisition sessions as a whole.
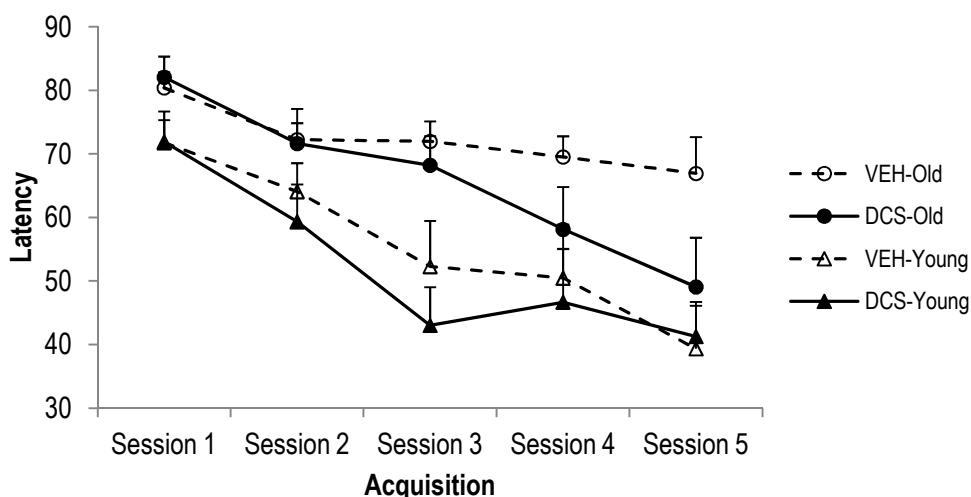
Figure 4. Latency in finding the hidden platform (s ±SE) during MWM acquisition for each group.

Concerning the control variables during acquisition (Table 2), there were no statistically significant *Age* effects ($F_{[1,36]}$ = 3.815, P = 0.059; $F_{[1,36]}$ = 3.154, P = 0.084), *Drug* effects ($F_{[1,36]}$ = 3.969, P = 0.054; $F_{[1,36]}$ = 1.984, P = 0.168) or *Age x Drug* interaction ($F_{[1,36]}$ = 0.123, P = 0.728; $F_{[1,36]}$ = 0.155, P = 0.696) in the variable *Thigmotaxis* and *Length*, respectively. In the variable *Speed*, there were *Age* ($F_{[1,36]}$ = 5.558, P = 0.024) and *Drug* ($F_{[1,36]}$ = 5.808, P = 0.021) statistically significant effects, but no *Age x Drug* interaction effects ($F_{[1,36]}$ = 1.791, P = 0.189), which revealed that the young rats swum faster than the old rats and that rats administered with PBS were faster than those administered with DCS.

Acquisition		VEH-Old	DCS-Old	VEH-Young	DCS-Young
	Session 1	52.65 ±11.1	52.5 ±7.7	52.6 ±10.5	52.4 ±14
	Session 2	54.41 ±10.9	49.79 ±12.4	53.43 ±18.4	43.33 ±17.7
Thigmotaxis	Session 3	52.41 ±10.5	41.94 ±14.3	41.48 ±16.8	33.56 ±13.5
	Session 4	46.46 ±9.9	38.43 ±20.1	39.47 ±21	30.72 ±14.4
	Session 5	44.73 ±14.8	30.75 ±13.6	27.1 ±9.5	28 ±16.1
	Session 1	37.87 ±2.5	38.37 ±4.3	46.41 ±6.3	42.32 ±5.6
	Session 2	42.6 ±4.6	41.84 ±5.3	49.87 ±8.3	45.13 ±4.6
Speed	Session 3	42.47 ±5.5	41.6 ±5.4	48.11 ±4.1	41.27 ±4.8
	Session 4	43.14 ±4	40.3 ±6.2	46.71 ±7	41.43 ±5.1
	Session 5	42 ±6.2	38.66 ±5.8	42.14 ±8.9	37.56 ±8.4
	Session 1	3803.34 ±602.2	3095.31 ±528.7	3098.47 ±686.9	2924 ±1043.1
	Session 2	2946.77 ±683	3025.53 ±507	3223.64 ±907.3	2700.57 ±870.8
Length	Session 3	2953.24 ±441.3	2846.1 ±784.4	2447.62 ±1125.7	1804.16 ±904.9
	Session 4	2885.4 ±401.8	2327 ±928	2348.61 ±1408.4	2000.45 1265.5
	Session 5	2622.77 ±817.9	1917.36 ±1037.8	1730.86 ±1381.2	1644.05 ±835.3

Table 2. Comparison between thigmotaxis (percentage ±SE), speed (cm/s ±SE) and length (cm ±SE) during acquisition for each group.

One of the variables analyzed during the probe test was the *Percentage of time in the target quadrant* and the ANOVA analysis revealed no statistically significant effects of *Age* ($F_{[1,36]}$ = 1.692, P = 0.202), *Drug* ($F_{[1,36]}$ = 1.279, P = 0.266) or *Age x Drug* ($F_{[1,36]}$ = 0.869, P = 0.357). The one-sample t test against a constant (25) showed that all groups performed above chance level (VEH-Old: $t_{(10)}$ = 3.12, P = 0.011; DCS-Old: $t_{(9)}$ = 3.628, P = 0.005; VEH-Young: $t_{(9)}$ = 3.203, P = 0.011; DCS-Young: $t_{(8)}$ = 4.374, P = 0.002) (Fig. 5A).

However, Figure 5B depicts that during the first 30 s all groups performed above the chance level (VEH-Old: $t_{(10)}$ = 3.868, P = 0.003; DCS-Old: $t_{(9)}$ = 3.148, P = 0.012; VEH-Young: $t_{(9)}$ = 3.566, P = 0.006; DCS-Young: $t_{(8)}$ = 2.756, P = 0.025), but during the second 30 s only the rats treated with DCS did (VEH-Old: $t_{(10)}$ = 1.499, P = 0.165; DCS-Old: $t_{(9)}$ = 2.824, P = 0.02; VEH-Young: $t_{(9)}$ = 1.596, P = 0.145; DCS-Young: $t_{(8)}$ = 3.916, P = 0.004).

Regarding the *Distance swum in the target quadrant* during the test, the analyses demonstrated statistically significant effects of *Age* ($F_{[1,36]}$ = 4.133, P = 0.049), but there were no significant effects of *Drug* ($F_{[1,36]}$ = 0.391, P = 0.536) or *Age x Drug* ($F_{[1,36]}$ = 0.402, P = 0.530), showing that the young rats swam a larger distance than the old rats within the target quadrant. Concerning the average *Distance to target*, there were statistically significant effects of *Age* ($F_{[1,36]}$ = 4.534, P = 0.04), although there were no *Drug* ($F_{[1,36]}$ = 2.938, P = 0.095) or *Age x Drug* ($F_{[1,36]}$ = 2.417, P = 0.129) effects, suggesting that the young rats travelled a shorter distance in order to reach the absent platform than the old rats.
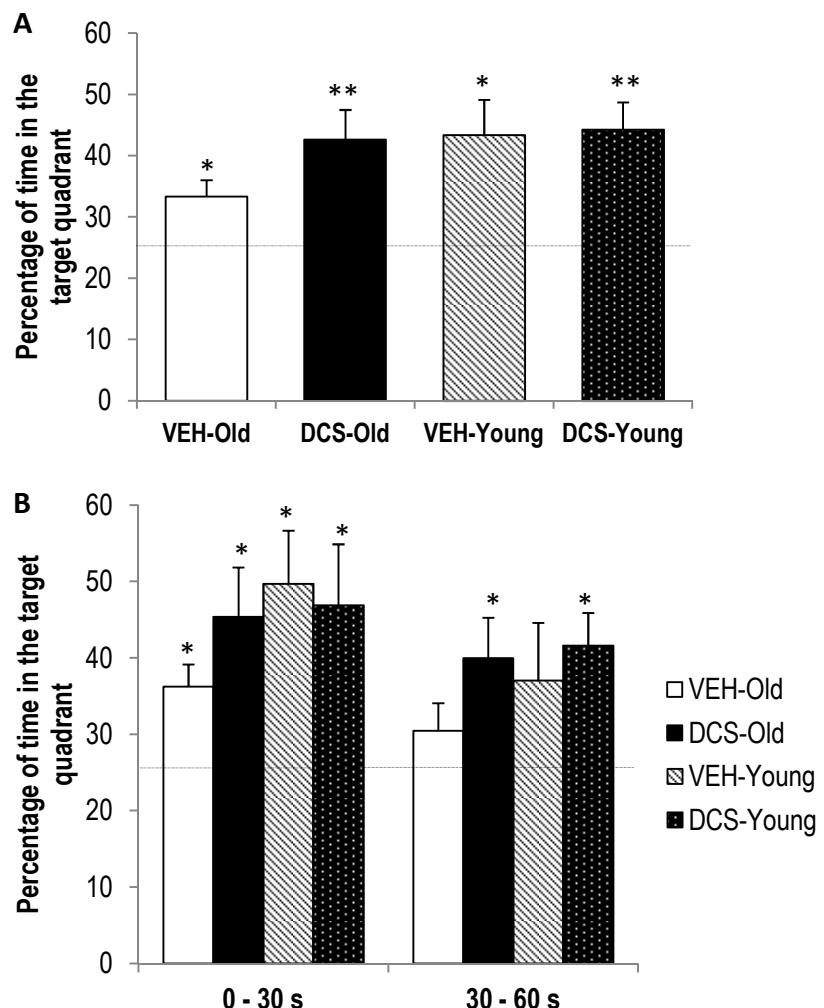
Figure 5. (A) Percentage of time spent in the target quadrant (±SE) during the MWM probe test for each group. (B) Percentage of time in the target quadrant (±SE) during the MWM probe test divided into the first 30 s and the second 30 s for each group. * P < 0.05 and ** P < 0.01 (regarding chance level).

During the reversal training, the statistical analyses demonstrated statistically significant effects of *Age* ($F_{[1,36]}$ = 12.926, P = 0.001) and *Age x Drug* interaction ($F_{[1,36]}$ = 5.681, P = 0.023) in the *Latency in finding the hidden platform* (Fig. 6), although *Drug* effect was not significant ($F_{[1,36]}$ = 2.149, P = 0.151). The contrast analysis showed that there were significant differences between VEH-Old and DCS-Old (P = 0.008) and VEH-Young (P < 0.0001).

Concerning the time spent swimming in the SE quadrant (where the platform was in the acquisition sessions), the analysis revealed a statistically significant effect of the *Age* in *Percentage of time spent in the SE quadrant* ($F_{[1,36]}$ = 6.413, P = 0.016) and in *Percentage of time spent in the SE target annulus* ($F_{[1,36]}$ = 4.849, P = 0.034). However, there were no statistically significant effects of *Drug* or *Age x Drug* in *Percentage of time spent in the SE quadrant (Drug*: $F_{[1,36]}$ = 0.239, P = 0.628; *Age x Drug*: $F_{[1,36]}$ = 0.263, P = 0.611), or in *Percentage of time spent in the SE target annulus (Drug*: $F_{[1,36]}$ = 0.637, P = 0.430; *Age x Drug*: $F_{[1,36]}$ = 0.025, P = 0.876).
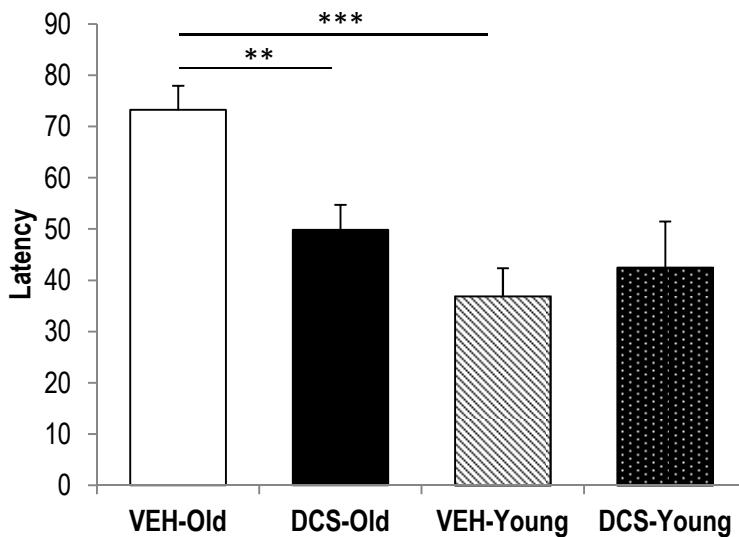
Figure 6. Latency in finding the hidden platform (s ±SE) during the MWM reversal learning for each group.
** P < 0.01 *** P < 0.0001

Regarding the control variables (Table 3), there were statistically significant *Age* effects in the test and reversal (Test: $F_{[1,36]}$ = 4.712, P = 0.037; Reversal: $F_{[1,36]}$ = 9.315, P = 0.004) in *Thigmotaxis*. Significant *Drug* effects were found in the test ($F_{[1,36]}$ = 4.849, P = 0.034) but not in the reversal ($F_{[1,36]}$ = 1.397, P = 0.245) and there were no statistically significant differences in the interaction *Age x Drug* in the test or reversal (Test: $F_{[1,36]}$ = 3.772, P = 0.06; Reversal: $F_{[1,36]}$ = 2.396, P = 0.13). When analyzed the *Length*, there were statistically significant effects of *Age* in the reversal ($F_{[1,36]}$ = 5.514, P = 0.024) but not in the test ($F_{[1,36]}$ = 0.12, P = 0.731). There were no significant *Drug* effects in the test ($F_{[1,36]}$ = 0.73, P = 0.399) and the reversal ($F_{[1,36]}$ = 0.666, P = 0.42) or *Age x Drug* effects (Test: $F_{[1,36]}$ = 0.07, P = 0.793; Reversal: $F_{[1,36]}$ = 3.129, P = 0.085). Finally, regarding the variable *Speed*, during test and reversal there were no *Age* (Test: $F_{[1,36]}$ = 0.118, P = 0.733; Reversal: $F_{[1,36]}$ = 0.876, P = 0.356), *Drug* (Test: $F_{[1,36]}$ = 0.71, P = 0.405; Reversal: $F_{[1,36]}$ = 0.009, P = 0.926) or *Age x Drug* significant effects (Test: $F_{[1,36]}$ = 0.063, P = 0.803; Reversal: $F_{[1,36]}$ = 0.004, P = 0.952).

		VEH-Old	DCS-Old	VEH-Young	DCS-Young
Test	**Thigmotaxis**	51.83 ±19.9	29.65 ±15.	29.82 ±18.4	28.43 ±11.8
	Speed	46.52 ±6.1	43.98 ±8.2	45.14 ±7.6	43.77 ±7.2
	Length	2790.2 ±369	2634.51 ±497	2705.25 ±453.3	2623.14 ±433.6
Reversal	**Thigmotaxis**	43.61 ±17	30.59 ±18.1	21.67 ±10.4	23.41 ±12.9
	Speed	38 ±4.3	38 ±6.4	39.67 ±5.8	39.97 ±7.6
	Length	2608.49 ±814.7	1853.6 ±590.3	1406.36 ±921.6	1684.51 ±1280.6

Table 3. Comparison between thigmotaxis (percentage ±SE), speed (cm/s ±SE) and length (cm ±SE) during test and reversal for each group.

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Finally, examples of swimming paths of each group are presented (Fig. 7). In the first day of acquisition, all groups showed thigmotactic behavior and a random search strategy. Progressively, all groups followed a more direct path to reach the hidden platform across the training days, although VEH-Old rats had more difficulties. Throughout the experiment, old rats tended to use a circling strategy (Gallagher, Burwell & Burchinal, 1993), which is less efficient than learning the location of the platform but it is better than a random search. Regarding the test, all groups significantly spent more time in the target quadrant and in the target annulus. However, although there were no significant between-group differences, the swimming paths revealed that VEH-Old rats did not focus their search in the target quadrant as much as the other groups. During reversal, young and DCS-Old rats were able to learn the new location, although both groups of old rats partly focused their search in the SE quadrant.
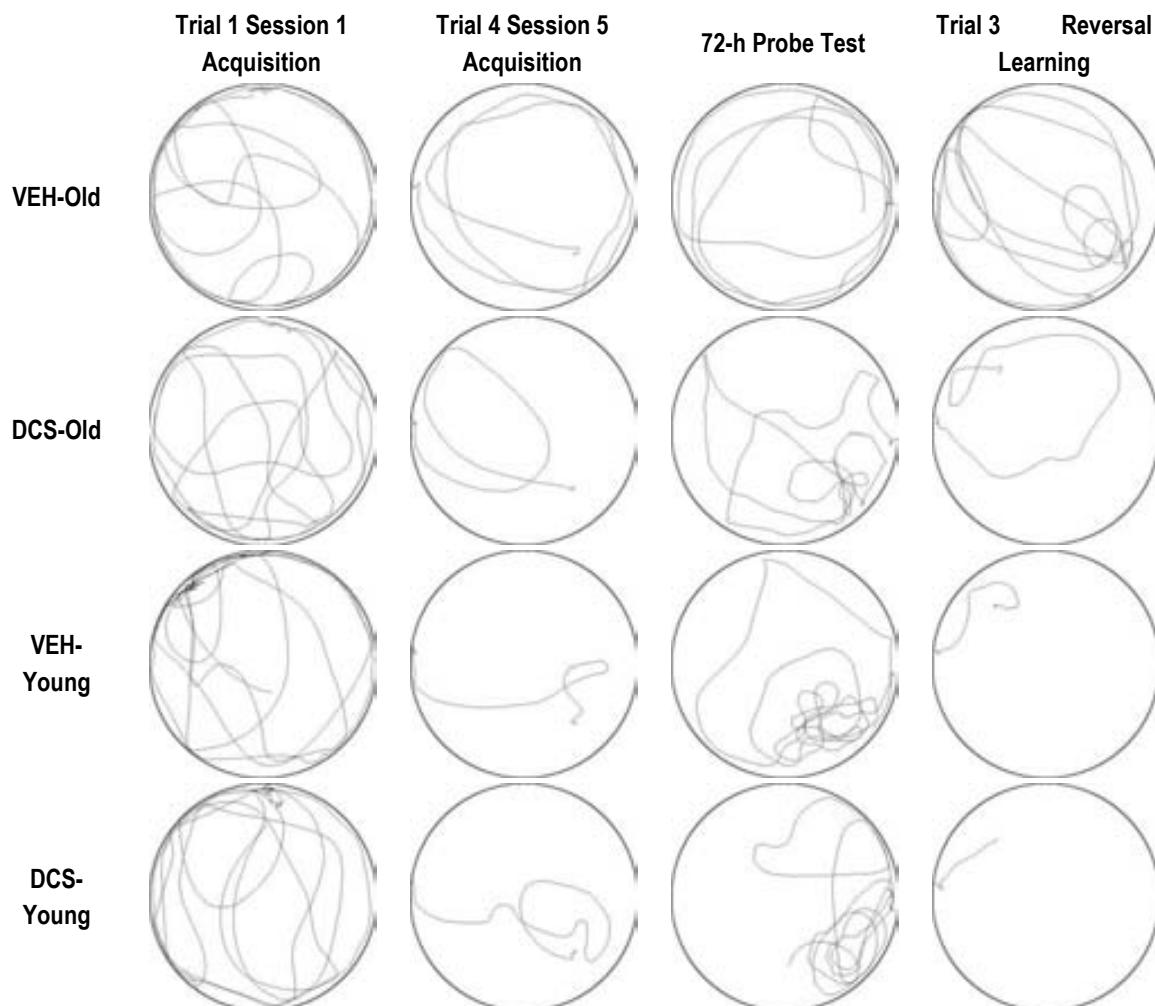
Figure 7. Swimming paths recorded during acquisition, probe test and reversal learning in order to illustrate the paths followed by a subject of each group.

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4. Discussion

The main objective of the present research was to determine whether the administration of DCS in the vHPC rescued the memory impairment due to aging of two hippocampal-dependent learning tasks, the STFP and the MWM. The results demonstrate, for the first time, that a single injection of DCS before STFP acquisition improved the drug-free 72-h olfactory memory test in old rats, while injections before each of the five MWM acquisition sessions failed to significantly improve the 72-h probe test but facilitated reversal learning. These results add more evidence to the role of DCS as a cognitive enhancer and suggest that boosting NMDARs function in the vHPC may improve certain cognitive functions in aged animals.

In the STFP task, the VEH-Old rats exhibited a lower percentage of trained food eaten in the test compared to the remaining groups and their performance was not significantly different from the chance level, thus demonstrating a poorer retention of the task. These results confirm a previous study showing that old rats exhibited a faster forgetting after STFP training than young rats (Countryman & Gold, 2007). Such deficits in aged rats were reverted in the present experiment with an intra-vHPC injection of DCS, as well as in a previous study DCS also ameliorated STFP deficits induced by scopolamine, a pharmacological model for cholinergic cognitive impairment (Portero-Tresserra et al., 2013). However, the administration of DCS into the vHPC did not improve STFP memory in young rats, as observed earlier (Portero-Tresserra et al., 2013), whereas it enhanced memory in a simpler odor discrimination task when injected into the PLC in young rats (Portero-Tresserra et al., 2013). Therefore, such findings suggest that the STFP paradigm is a learning model suitable for the study of cognitive deficits due to aging and that it may be appropriate to analyze the properties of cognitive-enhancing compounds.

The facilitative effects of DCS on olfactory memory cannot be attributed to non-mnemonic variables such as changes in social interaction, neophobia, motivation to eat or motor behavior as the injection of DCS into the vHPC did not modify any of those variables. However, between-group differences were found regarding age during the social interaction, in which old rats performed a lower number of sniffs than the young rats, in agreement with a previous study reporting that old rats spent less time in active social interaction (Salchner, Lubec & Singewald, 2004). Despite these differences, a stronger social interaction does not seem to be associated with increases in STFP learning since there were no statistically significant correlations between any of the social interaction measures and the preference for the trained food. With regard to neophobia, although aged rats ate less ground food than young rats in the rehabituation session, both groups showed a similar intake during the test, which suggest that old rats did not show a neophobic reaction towards the flavored food. Moreover, DCS did not alter olfactory sensitivity, but there was a significant age effect, meaning that old rats spent more time in finding the buried cookie in comparison with young rats, which may be due to a possible age-related decrease in olfactory sensitivity (Kraemer & Apfelbach, 2004). Therefore, such results suggest

that although olfactory deficits may account for impaired food preference in old rats, the administration of DCS does not seem to have improved STFP memory by enhancing the olfactory perception. It is interesting to note that although olfactory memory seems to be of particular interest in aging studies due to the fact that it is affected by age-related brain changes in humans and animals (Kovács, 2004), few studies have examined odor memory impairments in old rats (Gilbert et al., 2009).

The findings in the MWM task revealed that all groups gradually reduced their latency to locate the platform during acquisition, which is an indicator of spatial learning and memory (Gallagher, Burwell & Burchinal, 1993). Additionally, DCS-Old rats showed a level of performance similar to that of young rats in the last acquisition session, while the decrease in the latency of VEH-Old rats was not as pronounced. However, DCS did not significantly enhance the old rats' performance during the acquisition or the 72-h probe test, but instead improved the reversal learning when compared to VEH-Old rats. This is consistent with previous results showing that the systemic administration of DCS also enhanced the MWM reversal learning (Riekkinen, Ikonen & Riekkinen, 1998). Regarding the control variables in the MWM acquisition, young and old rats demonstrated comparable rates of thigmotaxis and length. However, there was a significant age effect on speed, similarly as reported in a variety of studies (Carter et al., 2002; Leite-Almeida et al., 2009; Novier et al., 2013; Zhang et al., 2012), which indicated that old rats swam slower than young rats. Therefore, as there was no significant effect of age on the variable path length, the longer latency observed in old animals during acquisition may reflect certain motor difficulties rather than learning deficits.

During the 72-h probe test, the spatial accuracy, which was determined by the time the animal spent searching the platform in the target quadrant (D'Hooge & De Deyn, 2001), showed that all rats significantly spent more time in the target quadrant, suggesting that that they had correctly learnt the task. Nevertheless, it seems that the untreated old rats performed the task slightly worse than the other groups as depicted by the fact that their percentage of time in the target quadrant was closer to the chance level in comparison with the remaining groups. Moreover, the analysis of the latency to find the platform separately in two 30-s blocks showed that the rats treated with DCS, regardless their age, spent more time in the original target quadrant during the second block in comparison with vehicle rats, suggesting that DCS may have induced a stronger consolidation of the task. Moreover, the results of the total distance swum in the target quadrant during the test and the length travelled from the starting point to the hidden platform, which are indicative of closeness to the goal (Gallagher, Burwell & Burchinal, 1993), showed that the young rats swam larger distances within the target quadrant and followed a shorter path than the old animals. Such an outcome may be interpreted as if the young animals showed a more active search strategy and a better memory of the platform location.

During the reversal learning of the MWM, escape latencies were significantly affected by both age and drug. Old rats treated with DCS injected before the acquisition sessions showed in the reversal

phase of the experiment latencies similar to those of young rats and were significantly shorter than those of the untreated old rats. However, both old groups significantly spent more time in the quadrant where the platform was originally located in comparison with young rats, revealing that difficulties in the reversal of an original spatial learning increase with age, which agrees with previous results (Leite-Almeida et al., 2009). Thus, reversal learning, described as the ability to rapidly modify responses in order to adapt to changing task demands, involves behavioral and cognitive flexibility (Thong-asa et al., 2012) and declines with age with a shift towards increased cognitive rigidity (Nieves-Martinez et al., 2011). However, in the current experiment, although both old groups showed cognitive rigidity in some measure, only the DCS-Old rats were able to correctly learn the new location of the hidden platform. This suggests that the administration of DCS into the vHPC may enhance cognitive flexibility in old animals, i.e. the ability to rapidly adapt established goal-directed behavior when confronted with a changing goal.

Concerning the control variables during reversal learning, old rats travelled a larger path length, but there were no significant age differences in the speed, which suggests that the increases in latency described above cannot be attributable to motor disabilities but rather to learning difficulties, supporting the possibility of cognitive rigidity (Nieves-Martinez et al., 2011). With regard to thigmotactic behavior, a measure indicative of fearfulness (Von Lubitz et al., 1993) and poor search strategy (Brothers et al., 2013), old rats spent more time swimming near the walls during test and reversal learning, as it has already been reported in prior investigations (Burger et al., 2007; Brothers et al., 2013). Such stress response may have prevented them from adopting an accurate spatial search strategy and is consistent with the fact that old animals showed longer path lengths during reversal learning. However, as DCS-treated animals, regardless their age, showed a reduced path length during the probe test, we cannot reject the possibility that DCS enhanced memory in old rats by reducing stress levels, which agrees with the observation that systemic DCS is able to alleviate difficulties in learning and memory due to stress (Kart-Teke et al., 2006; Waddell et al., 2010; Yamamoto et al., 2008). However, these effects were not maintained at the reversal leaning, as the DCS enhanced the performance in old animals without affecting thigmotatic behavior.

As for the region involved in the facilitative impact of DCS, to our knowledge there are no previous studies investigating the effects of the DCS administration into the vHPC in aged animals. However, recent data have demonstrated that DCS injected in the vHPC rescued STFP memory and synaptic plasticity deficits induced by scopolamine in young rats (Portero-Tresserra et al., 2013). Furthermore, early research in young rats showed improvements in spatial working memory deficits, induced by the blockade of glutamatergic or muscarinic receptors, with injections of DCS in the dHPC (Kishi, Ohno & Watanabe, 1998; Ohno & Watanabe, 1996). Nevertheless, the comparison of the results obtained in both tasks in the current research leads us to consider some important aspects as

DCS into the vHPC was able to noticeably enhance STFP retention but not a MWM memory

test. A possible explanation could be that, although the vHPC is a critical region for STFP, as it has been demonstrated previously (Carballo-Márquez et al., 2009; Portero-Tresserra et al., 2013), it would not be the most appropriate area to inject DCS in order to improve MWM consolidation (Ferbinteanu, Ray & McDonald, 2003). Indeed, this task has been more linked to the dHPC since it seems more closely related to spatial or contextual learning (Bannerman et al., 2004). However, the vHPC seems more sensitive to the age-associated decline of NMDARs (Liu, Smith & Darlington, 2008; Magnusson, Kresge & Supon, 2006), which has also been related to spatial memory (Ferbinteanu, Ray & McDonald, 2003; Loureiro et al., 2012). Another possible explanation may be that DCS was administered acutely prior to the single STFP acquisition, while it was infused five consecutive days in the MWM training. Previous studies reported that systemic chronic administration of DCS would lead to desensitization and, consequently, to a reduction in responsiveness to the drug (Quartermain et al., 1994), which may reduce its capacity to act as a cognitive enhancer. A third hypothesis is that DCS may preferentially improve socially reinforced learning, as previous studies posited that its effects may be mediated by oxytocin and hence may facilitate social skills learning (Modi & Young, 2011; Otto, 2011). Finally, aged rats followed a caloric-restricted diet since they were four months old, a procedure that may have potentially contributed to reduce the age-related cognitive decline. In this regard, it is established that although caloric restriction has beneficial effects on health (Heilbronn & Ravussin, 2003), there are controversies regarding its effects on cognition (Gallagher, Stocker & Koh, 2011). However, preceding studies reported that caloric restriction delayed the impairments of age specifically in the MWM in comparison with *ad libitum* rats (Adams et al., 2008; Carter et al., 2009; Geng et al., 2007; Stewart, Mitchell & Kalant, 1989). Accordingly, in the present research, it may be interpreted that caloric restriction may have delayed age-deficiencies in the MWM test but not in the STFP test.

In relation to the mechanisms through which DCS may recover age-dependent cognitive deficits, it is widely accepted that the activation of the NMDARs is necessary for the enhancement of memory formation (Collingridge et al., 2013; Stern & Alberini, 2013). Indeed, there are age-related alterations in hippocampal synaptic plasticity mechanisms, including long-term potentiation (Barnes, 2003), and NMDARs activation (Potier et al., 2000). Therefore, since DCS is a partial NMDARs agonist that enhances synaptic plasticity in young and aged animals (Billard & Rouaud, 2007), the facilitative effects found here may be interpreted in terms of potentiation of synaptic plasticity (Portero-Tresserra et al., 2013; Rouaud & Billard, 2003).

In summary, the present results confirm that the positive modulation of glutamatergic transmission, acting on the glycine site of NMDA receptors, may be a good strategy for the study of the neural mechanisms responsible for the cognitive decline that appears with aging and provide an effective approach to test new treatments aimed at alleviating memory deficits.

5. Conclusions

DCS rescued the cognitive decline due to age in the STFP and the MWM reversal learning, but did not enhance the MWM test. The present investigation studied in old rats the effects of DCS administered in the vHPC, a structure markedly affected in Alzheimer's disease patients, a pathology that appears in a context of cognitive and neurobiological aging. Since the number of elderly is progressively increasing, there is growing interest on understanding how aging is related to cognitive decline. The use of naturally aged animals, in which memory and cognitive ability decrease due to senescence, can help us to understand the aging process because they model age-associated conditions in humans, like dementia. Moreover, such animal models are important for the development of therapeutic strategies that may lead to improved cognitive capabilities.

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V. Discussió

V. Discussió

L'objectiu principal d'aquesta tesi doctoral ha estat investigar la capacitat de la DCS, fàrmac agonista dels rNMDA que actua al lloc d'unió de la glicina i facilita la transmissió glutamatèrgica, com a potenciador dels processos d'aprenentatge i memòria. Ens hem plantejat si l'administració intracerebral podria ser capaç de revertir els dèficits cognitius associats al bloqueig muscarínic o al procés d'envelliment i hem analitzat els seus mecanismes d'acció a nivell cel·lular. La transmissió sinàptica glutamatèrgica a través dels rNMDA està implicada en els processos d'aprenentatge i memòria (Lee & Silva 2009) i, per aquest motiu, els fàrmacs que actuen potenciant l'activitat d'aquests receptors han estat considerats com a possibles tractaments farmacològics de diverses patologies neurològiques amb l'objectiu de millorar els processos cognitius (Collingridge et al. 2013). Si bé la transmissió glutamatèrgica és clau per a la formació de la memòria (Mayford et al. 2012), també s'ha descrit que interactua amb altres sistemes de neurotransmissió els quals poden modular la seva resposta. Específicament, s'ha demostrat que existeix una interacció entre els rNMDA i els receptors colinèrgics muscarínics en la regulació de l'aprenentatge i la memòria (Figuererdo et al. 2008). Tot i així, es necessita una millor comprensió de les estructures cerebrals on té lloc aquesta interacció i quins mecanismes neurals estan involucrats en aquest procés. En aquest context, per tal d'investigar la funció de la DCS com a potenciador cognitiu, en el present treball es van seleccionar regions cerebrals que formen part dels circuits neuroanatòmics subjacents a diverses formes d'aprenentatge i memòria com són l'ABL, el CPL i l'HPCv. La DCS va ser injectada preentrenament i els seus efectes van ser avaluats en proves de memòria olfactòria de tipus associatiu o relacional i en la plasticitat neural després d'haver bloquejat els receptors muscarínics amb l'administració d'SCOP. A més, també es van analitzar els efectes de la DCS en la memòria relacional olfactòria i espacial en animals de 24 mesos.

Els resultats principals del present treball han posat de manifest que la DCS a l'ABL va actuar com un potenciador dels processos d'aprenentatge i memòria, retardant l'extinció i facilitant la reconsolidació d'un aprenentatge de discriminació olfactòria, la DSO. La DCS al CPL també va permetre revertir els dèficits suscitats pel bloqueig de la transmissió colinèrgica en dues tasques basades en estímuls olfactoris, la DSO i la TSPA, confirmant així la interacció dels mecanismes glutamatèrgics i colinèrgics en aquesta regió cortical en la modulació de la memòria. Tanmateix, la DCS a l'HPCv va revertir el dèficit mnemònic observat a la TSPA induït per l'SCOP i va millorar el deteriorament de la memòria olfactòria i espacial observat en animals vells. Finalment, sembla que

aquests efectes de facilitació podrien estar mediats per una millora en el manteniment de la plasticitat sinàptica i concretament per la capacitat d'enfortir la PLT.

La D-cicloserina retarda l'extinció i facilita la reconsolidació de la memòria olfactòria

Els resultats obtinguts en el primer treball (Experiment 1) van demostrar que l'administració de DCS a l'ABL abans de l'extinció d'una tasca simple de discriminació olfactòria, com és la DSO, deteriorava tant l'adquisició com la retenció a les 24 hores del nou aprenentatge. En concret, el grup que va rebre DCS va mostrar dificultats per extingir la resposta condicionada, ja que presentava en les sessions d'extinció latències més curtes en trobar l'estímul reforçat, un menor nombre d'errors i un major nombre d'assajos per assolir el criteri d'extinció. En la sessió de retenció, per exemple, només el 66,5% dels animals amb DCS havien adquirit el criteri en l'últim assaig en comparació amb un 100% dels animals del grup VEH. De forma contrària, les rates control eren capaces de formar una nova traça de memòria que codificava la dissociació entre els estímuls després d'algunes exposicions consecutives a l'olor reforçada en absència de la recompensa. La resistència a l'extinció observada en el grup DCS també es va observar en la readquisició de l'aprenentatge original efectuat després de l'entrenament d'extinció, la qual va ser més ràpida que en el grup control. Per tant, els resultats semblaven indicar que els animals tractats amb DCS a l'ABL mostraven resistència a l'adquisició del nou aprenentatge d'extinció, efecte que podria interpretar-se com un enfortiment de la memòria original.

Les dades obtingudes en aquest experiment no coincideixen amb les observades en l'extinció del condicionament de la por, però sí que concorden amb les dels estudis d'administració sistèmica de DCS preentrenament, els quals no troben facilitació de l'extinció de tasques apetitives reforçades amb menjar (Port & Seybold 1998; Vurbic et al. 2011) o amb alcohol (Groblewski et al. 2009). D'altra banda, estudis recents en humans també han posat de manifest que l'administració de DCS abans del tractament d'extinció de la dependència de drogues no va tenir efectes notoris (Watson et al. 2011) i, fins i tot, va incrementar el desig pel seu consum (Price et al. 2011). Amb tot, observem certes diferències entre el nostre experiment i les investigacions prèvies que han observat millora de la consolidació de l'extinció. Per exemple, la DCS s'administra de manera sistèmica després de l'aprenentatge apetitiu operant (Leslie et al. 2012; Shaw et al. 2009) i sistèmica o intra-ABL després del condicionament pel lloc reforçat amb drogues (Paolone et al. 2009; Thanos et al. 2009). D'aquesta manera, el moment de l'administració del fàrmac, abans o després de l'adquisició, sembla ser una variable important en l'extinció de tasques reforçades amb estímuls apetitius. Tot i això, altres factors també podrien contribuir a explicar les diferències observades com la durada de l'entrenament (Flavell et al. 2011), el nivell d'ansietat dels subjectes (Tomilenko & Dubrovina 2007) o

el nivell d'extinció adquirit prèviament (Bouton et al. 2008). Pel que fa al temps d'exposició a l'estímul capaç de reactivar la memòria, és a dir, la durada de l'entrenament d'extinció, s'ha suggerit que una exposició breu a l'EC en absència de l'EI pot conduir a un procés de reconsolidació de la memòria i no a l'extinció de la tasca apresa (Lee et al. 2006). Així doncs, si bé el protocol utilitzat en la nostra recerca semblava suficient, ja que el grup VEH va mostrar signes marcats d'extinció, l'administració de DCS podria haver retardat el procés d'extinció evitant la formació d'un nou aprenentatge o bé enfortit la memòria original. Aquesta hipòtesi es va poder confirmar amb els resultats del segon treball (Experiment 2), en el qual es va administrar DCS a l'ABL abans d'una breu reactivació de la memòria. Els resultats van mostrar que aquest procediment facilitava la reconsolidació de la DSO a les 24 hores. En aquest estudi, els subjectes que van rebre la infusió de DCS abans de ser exposats breument a l'EC van mostrar una millora en la sessió de retenció, tant en les latències de resposta com en el nombre d'errors, en comparació amb els animals únicament que havien reactivat la memòria, els que només havien rebut la infusió de DCS o bé els de control.

Així doncs, les dades obtingudes en l'experiment 2 confirmen la hipòtesi que la reactivació de la memòria pot ser un mecanisme útil per enfortir la traça de la memòria original a través de l'administració de DCS. Si bé en aquest treball es va estudiar per primera vegada els efectes de la DCS en la reconsolidació d'una tasca apetitiva que utilitza reforçadors naturals, l'administració de DCS a l'ABL també exerceix efectes positius en la reconsolidació de la memòria de la preferència pel lloc associat amb cocaïna (Lee et al. 2009). De la mateixa manera, en paradigmes aversius també s'ha demostrat que la DCS, injectada per via sistèmica o a l'ABL, potencia la reconsolidació de la memòria de la por (Lee et al. 2006; Yamada et al. 2009). A més a més, un estudi previ va demostrar que el bloqueig dels rNMDA després de la reactivació de la memòria impedia la reconsolidació de la DSO (Torras-Garcia et al. 2005), resultat que reforça la hipòtesi que la potenciació dels rNMDA pot ajudar a estabilitzar la traça de memòria original d'aquesta tasca. En el nostre experiment la DCS es va administrar abans de presentar l'EC, fet pel qual la DCS podria haver modulat la reactivació de la memòria *per se* i no la reconsolidació. Però de forma similar, el bloqueig dels rNMDA de l'ABL abans de la reactivació dificulta la reconsolidació de la memòria (Lee et al. 2008). No obstant això, amb l'objectiu d'aïllar de forma més específica els efectes de la DCS en la reconsolidació de la memòria, seria adient realitzar estudis que analitzin els efectes de les injeccions de DCS postreactivació.

Finalment, malgrat que els mecanismes d'acció específics pels quals la DCS actua com un potenciador cognitiu no són del tot coneguts (Davis et al. 2006), s'ha descrit que la plasticitat sinàptica en l'ABL podria ser un factor determinant en l'aprenentatge d'extinció i la reconsolidació dels records emocionals (Duvarci et al. 2006; Myers & Davis 2002). Per tant, l'augment de l'eficàcia dels rNMDA induïda per l'administració de DCS podria promoure un seguit de canvis intracel·lulars

que generarien modificacions duradores de la transmissió sinàptica a les neurones corticals on projecta l'ABL (Mao et al. 2008). Per últim, si bé l'ABL és la regió cerebral més involucrada en l'extinció i la reconsolidació de diferents tasques, altres estructures, com l'HPC (Nader & Hardt 2009) i el CPF (Quirk & Mueller 2008), també podrien participar en aquests processos.

En resum, els resultats d'aquests dos primers experiments mostren que la potenciació de l'activitat glutamatèrgica a l'ABL mitjançant l'administració de DCS millora processos d'aprenentatge i memòria d'una tasca de discriminació olfactòria, ja que sembla enfortir la persistència de la traça de memòria original. Aquest fet es veu demostrat per una resistència en l'extinció i una facilitació de reconsolidació i de la readquisició de la tasca.

La D-cicloserina reverteix els dèficits de memòria olfactòria produïts pel bloqueig de la transmissió colinèrgica

Considerant el fet que la DCS és un fàrmac que pot potenciar els processos de memòria i que sembla revertir dèficits mnemònics produïts per patologies o manipulacions farmacològiques (Dhawan 2011), els experiments 3, 4 i 5 del present treball van estudiar si l'administració intracerebral a regions involucrades en l'aprenentatge, com el CPL (Experiments 3 i 4) i l'HPC (Experiment 5), podia revertir els dèficits en la memòria olfactòria resultants del bloqueig de la transmissió muscarínica. La memòria olfactòria depèn especialment del correcte funcionament de l'HPC i del CPF i s'ha vist alterada tant per antagonistes dels rNMDA com per l'administració d'SCOP (Roberts & Shapiro 2002; Carballo-Marquez 2009). A més, l'olfacte consisteix en el sentit més primitiu filogenèticament i el més sensible per guiar la conducta dels rosegadors. Per aquest motiu l'ús de tasques d'aprenentatge olfactori són un bon model per avaluar la interacció entre les vies glutamatèrgiques i colinèrgiques en la modulació de la memòria.

Els resultats dels experiments 3 i 4 van mostrar que la potenciació de la funció del rNMDA en el CPL, mitjançant l'administració de DCS, va atenuar els dèficits provocats per l'SCOP en la memòria olfactòria avaluada en dos paradigmes apetitius, un de discriminació olfactòria (DSO) i un altre de tipus social (TSPA). Aquests resultats no poden ser atribuïts a variables no cognitives com són l'alteració de la percepció olfactòria, la interacció social entre animals, la resposta de neofòbia o l'activitat motora, ja que l'anàlisi estadística no va mostrar diferències entre els animals tractats amb DCS i/o SCOP i els VEH. Així doncs, la DCS sembla compensar els dèficits cognitius produïts pel bloqueig muscarínic, el que coincideix amb les dades obtingudes en altres paradigmes d'aprenentatge (Andersen et al. 2002). Les dades també repliquen els efectes negatius sobre la memòria de la DSO i la TSPA produïts per l'SCOP intracerebral (Boix-Trelis et al. 2007; Carballo-Marquez et al. 2009a) i estan a favor de considerar l'administració d'SCOP com un model

farmacològic de dèficit cognitiu (Klinkenberg & Blokland 2010). Amb tot, l'efecte de reversió de la DCS va ser més notable en la DSO, on l'execució del grup DCS+SCOP durant el test de memòria no es va diferenciar del grup VEH. En la TSPA, en canvi, el grup DCS+SCOP va demostrar un millor record que el grup SCOP però no es va igualar al grup control en el test de preferència alimentària. A més a més, les dades de l'experiment 3 van suggerir que una sola injecció de DCS al CPL abans de l'adquisició millorava l'execució de la DSO en rates no tractades amb SCOP. Així, els animals del grup DCS van mostrar latències més baixes i un menor nombre d'errors que els animals VEH en el test de retenció a les 24 hores. Aquests resultats repliquen estudis anteriors que indiquen que les rates tractades amb DCS obtenen un millor rendiment en la retenció de la DSO (Villarejo-Rodriguez et al. 2010) i corroboren que els rNMDA del CPL modulen la formació de la memòria d'aquesta tasca (Tronel & Sara 2003). El fet que la DCS només afectés la prova de retenció, sense produir efectes durant l'adquisició, descarta la possibilitat d'una situació d'aprenentatge dependent de l'estat. En la TSPA, a diferència de la DSO, la modulació positiva dels rNMDA al CPL amb DCS no va millorar la memòria en rates control, el que contrasta amb estudis previs que mostren que el bloqueig dels rNMDA provoca efectes amnèsics en aquesta tasca (Burne et al. 2010; Roberts & Shapiro 2002).

Així doncs, els resultats provinents de l'experiment 3 semblen indicar que la DCS podria exercir efectes diferents en funció del paradigma d'aprenentatge utilitzat, ja que millora tasques de memòria associativa de tipus implícit com la DSO, però sembla tenir limitacions per facilitar tasques relacionals i socials com la TSPA. Si bé els estudis previs corroboren que la DCS afavoreix tasques de memòria implícita tant en animals com en humans (Walker et al. 2002; Nunnink et al. 2007; Kuriyama et al. 2011), sense produir efectes en tasques de memòria espacial en animals (Sunyer et al. 2008) o declarativa en humans (Kuriyama et al. 2011), altres estudis suggereixen que la DCS podria també ser eficient en paradigmes hipocamp dependents (Assini et al. 2009; Onur et al. 2010). Una interpretació addicional dels resultats descrits podria ser que, tot i que el CPL participa en la consolidació de la memòria de la TSPA (Smith et al. 2007), aquesta regió cerebral s'ha vist especialment implicada quan el disseny de la TSPA suposa una major exigència cognitiva i en el test de retenció s'introdueix una tercera opció de resposta (Winocur & Moscovitch 1999). A més a més, el fet de presentar tres alternatives permet ampliar el rang del percentatge de preferència alimentària enregistrat i, en el cas que s'hagués produït un efecte sostre durant l'experiment 3, es podria observar un possible efecte potenciador de la DCS. Aquesta hipòtesi es va poder contrastar gràcies als resultats de l'experiment 4, en el qual es va avaluar l'efecte dels dos fàrmacs, DCS i SCOP, quan la TSPA va augmentar el nivell de dificultat. Els resultats van indicar que en aquest paradigma la DCS al CPL va ser capaç de revertir els dèficits derivats a l'SCOP i els animals del grup DCS+SCOP no van diferenciar-se significativament dels animals del grup VEH. No obstant això, en aquest estudi, la

infusió de DCS per si mateixa tampoc va enfortir la memòria de la TSPA. Amb tot, podem considerar altres factors per explicar la mancança d'efectes contundents de la DCS intraCPL en la TSPA. Per exemple, una dosi més alta podria haver proporcionat efectes potenciadors, tal com s'ha descrit en altres paradigmes (Zlomuzica et al. 2007), però cal tenir present que la DCS mostra una corba dosi-resposta en forma d'U invertida (Ozawa et al. 2012), així que un increment de la dosi no necessàriament es veuria acompanyat d'una millora de la memòria i inclús podria produir efectes no desitjats. Per últim, altres àrees cerebrals, a més del CPL, podrien ser més sensibles a l'administració intracerebral de DCS, com ara la formació hipocampal, la qual ha estat clarament implicada en la consolidació de la TSPA (Alvarez et al. 2001) així com en altres tasques de memòria relacional (Eichenbaum 1999).

L'experiment 5 va avaluar si la DCS administrada a la formació hipocampal podria revertir el dèficit de memòria de la TSPA produït per l'SCOP i millorar l'aprenentatge de la tasca en rates no tractades. Els resultats principals d'aquest experiment van evidenciar que l'augment de l'activitat glutamatèrgica a la regió ventral de l'HPC va minimitzar el dèficit de memòria induït per la injecció d'SCOP. Així, els animals del grup DCS+SCOP van mostrar una preferència alimentària diferent del nivell d'atzar i del grup SCOP, i similar a la del grup VEH. Aquestes dades confirmen estudis anteriors i suggereixen que l'HPC pot ser una regió crítica per a la interacció entre la DCS i l'SCOP en la modulació de la memòria (Ohno & Watanabe 1996; Kishi et al. 1998), coincidint amb els resultats obtinguts amb administracions sistèmiques d'aquests fàrmacs (Andersen et al. 2002; Pitkänen et al. 1995). D'altra banda, la injecció d'SCOP a l'HPCv després de l'adquisició de la TSPA va produir un dèficit notable en la prova de retenció a les 24 hores, ja que el grup SCOP no va mostrar diferències respecte al nivell d'atzar i la seva preferència alimentària va ser substancialment inferior a la del grup VEH. Aquestes dades corroboren la contribució dels receptors muscarínics en la consolidació de la memòria de la TSPA (Carballo-Màrquez et al. 2009a) i en altres tasques hipocamp dependent (Wallenstein & Vago 2001). No obstant això, en aquest experiment tampoc es va observar un efecte facilitador de la memòria de la TSPA amb l'administració de DCS en animals no tractats, consistent amb les dades dels experiments 3 i 4. Cal tenir present que és la primera vegada que s'investiguen els efectes de l'administració intracerebral de DCS en la consolidació de la memòria de paradigmes dependents d'hipocamp i els estudis anteriors realitzats amb administracions sistèmiques mostren discrepàncies en els resultats obtinguts (Lelong et al. 2001; Sunyer et al. 2008).

La D-cicloserina reverteix els dèficits de memòria induint plasticitat neural

Amb l'objectiu d'investigar els mecanismes d'acció de la DCS per revertir els efectes de l'SCOP a nivell cel·lular es va realitzar un estudi electrofisiològic en seccions d'hipocamp de rata (Experiment 6). Els resultats del treball van mostrar, per primera vegada, que els mecanismes de plasticitat sinàptica en sinapsis de CA1 estan involucrats en les propietats compensatòries d'aquests dos fàrmacs. Així doncs, el manteniment de la PLT va ser afectat negativament pel bloqueig muscarínic i va ser restaurat amb la perfusió paral·lela de dues dosis diferents de DCS. Aquestes dades concorden amb l'efecte facilitador de la PLT promogut per la DCS en animals amb lesions traumàtiques (Yaka et al. 2007), deficients d'NCAM (Kochlamazashvili et al. 2012) o animals envellits (Billard & Rouaud 2007). Les dades electrofisiològiques coincideixen amb els efectes observats en treballs anteriors on la infusió d'SCOP havia afectat la PLT hipocampal (Hirotsu et al. 1987; Ye et al. 2001; Sánchez et al. 2009; Ovsepian et al. 2004) i confirmen la participació dels receptors muscarínics en la plasticitat neural a través de la modulació dels rNMDA (Boddeke et al. 1992; Buchanan et al. 2010). D'altra banda, en aquest experiment també es va mostrar que l'administració de DCS a CA1 abans de la inducció de PLT augmentava la magnitud dels PEP registrats. No obstant això, si bé aquests resultats semblen replicar estudis previs (Rouaud & Billard 2003), en el present estudi la DCS per si mateixa no va potenciar el manteniment de la PLT en seccions no tractades amb SCOP.

Els resultats dels experiments anteriors semblen suggerir una possible interacció funcional entre els sistemes colinèrgic i glutamatèrgic en la modulació dels processos de memòria tant a nivell conductual com cel·lular. Aquesta interacció podria produir-se en regions cerebrals com el CPL i l'HPCv, on el bloqueig de l'activitat dels receptors muscarínics podria ser compensat amb l'activació dels rNMDA per tal de regular diferents processos cognitius i les seves bases sinàptiques subjacents. Les presents dades són congruents amb estudis previs que mostren que l'activació dels receptors muscarínics de l'HPC amplia els PEP modulant l'activitat dels rNMDA (Markram & Segal 1990) i augmenta la probabilitat d'induir PLT. De fet, l'activació dels receptors colinèrgics ha de coincidir amb l'alliberació de Glu per induir canvis plàstics de llarga durada (Navakkode & Korte 2012). Si bé aquests resultats han estat descrits bàsicament a l'HPC, també podrien tenir lloc a regions neocorticals, com el CPF. En aquest sentit s'ha observat que els rNMDA i els receptors colinèrgics modulen conjuntament el funcionament electrofisiològic de les cèl·lules corticals (Greuel et al. 1988; Dijk et al. 1995). A més, aquesta interacció també s'observa en estudis conductuals en els quals l'administració de dosis inefectives d'SCOP junt amb antagonistes dels rNMDA, a l'HPC o de forma sistèmica, provoca efectes amnèsics en diferents tasques (Hlinák & Krejcí 1998; Khakpai et al. 2012).

En resum, els nostres resultats demostren que l'administració de DCS va ser capaç de revertir els dèficits causats per l'SCOP tant a nivell conductual, recuperant el deteriorament de la memòria, com cel·lular, potenciant el manteniment de la PLT. Per tant, podem concloure que la DCS reverteix dèficits de memòria produïts per la depleció colinèrgica probablement induint canvis en la plasticitat sinàptica i augmentant, per tant, la força entre les connexions sinàptiques.

La D-cicloserina reverteix dèficits de memòria produïts pel procés d'envelliment

L'últim experiment (Experiment 7) de la present tesi doctoral va tenir com a objectiu investigar els possibles efectes beneficiosos de la DCS injectada a la formació hipocampal per tal de reduir els dèficits cognitius associats a l'envelliment normal. Per tal de realitzar aquest estudi vam utilitzar rates de 24 mesos i vam avaluar la seva memòria en tasques relacionals hipocamp dependents, una de naturalesa olfactòria i social, com és la TSPA, i una altra de memòria espacial, com és el LAM. Els resultats d'aquest experiment van revelar que la injecció de DCS a l'HPCv abans de l'adquisició de la TSPA millorava la prova de memòria mesurada 72 hores després. Així, les rates velles tractades amb DCS no es van diferenciar de les rates joves pel que fa a la preferència alimentària, en canvi, les rates velles sense tractar van presentar un rellevant dèficit de memòria. Aquests resultats concorden amb els obtinguts en un estudi previ, en el qual les rates velles van mostrar problemes en la retenció d'aquesta tasca (Countryman & Gold 2007). Els efectes de millora de la DCS en la memòria olfactòria de les rates velles no es poden atribuir a canvis en la sensibilitat olfactòria, la interacció social, la neofòbia, la motivació pel menjar o la conducta motora, ja que l'administració d'aquest fàrmac no va modificar aquestes variables. No obstant això, sí que es van observar diferències segons l'edat en la interacció social, ja que els animals vells van realitzar un menor nombre de contactes en comparació amb els animals joves. Aquestes dades coincideixen amb resultats previs, que van mostrar una disminució en la conducta social activa durant l'envelliment (Salchner et al. 2004). Amb tot, el nivell d'interacció social de les rates no va correlacionar amb la preferència alimentaria durant el test. També es va demostrar que les rates velles tenien una lleugera alteració en la sensibilitat olfactòria i van necessitar un major temps per trobar la galeta enterrada durant el test de sensibilitat olfactòria en comparació amb els animals joves. Si bé això podria explicar els problemes de memòria observats en les rates velles, la DCS no va afectar la sensibilitat olfactòria dels animals i, per tant, la millora observada en el grup de rates velles tractades amb DCS no pot ser atribuïda a un increment en la percepció dels estímuls olfactoris. Les tasques de memòria olfactòria podrien tenir un interès particular per a la investigació dels potenciadors cognitius, ja que en humans, durant l'envelliment, es poden donar problemes en la detecció d'olors que poden correlacionar amb l'inici d'una malaltia neurodegenerativa (Kovács 2004).

Els resultats obtinguts amb la tasca del LAM van ser menys notables, en aquest cas l'administració de DCS a l'HPCv abans de cada sessió d'entrenament no va millorar la prova de retenció al cap de 72 hores en les rates velles, tot i que sí que va ser capaç de facilitar l'aprenentatge de reversió. Així doncs, les dades experimentals van mostrar que durant l'adquisició de la tasca les rates velles, amb independència de si havien estat tractades o no, tardaven més a trobar la plataforma, ja que necessitaven recórrer més distància i nedar més lentament que els animals joves. Amb tot, en la prova de retenció no es van observar diferències estadísticament significatives entre grups. Els efectes beneficiosos de la DCS van ser observats a la prova de reversió, on els animals havien d'aprendre a trobar de nou la plataforma ubicada en un quadrant diferent de l'original. En aquesta prova, els animals DCS-Old van reaprendre la tasca de manera similar a les rates joves, resultats que van ser consistents amb estudis previs (Riekkinen et al. 1998). Així doncs, sembla que la DCS va millorar la flexibilitat cognitiva en les rates velles, malgrat que tots els animals envellits van mostrar un major percentatge de temps en el quadrant on es trobava la plataforma original que les rates joves. En relació amb les variables de control, les rates joves i velles van mostrar taxes comparables de tigmotaxis i distància recorreguda durant l'adquisició, però, com en altres experiments, els animals vells van mostrar una menor velocitat natatòria (Leite-Almeida et al. 2009; Novier et al. 2013). En la prova de test, les rates del grup DCS-Old van realitzar una menor conducta de tigmotaxis que les VEH-Old, la qual es pot considerar un indicatiu dels nivells d'estrès. Aquests resultats podrien suggerir que la DCS hauria reduït les dificultats d'aprenentatge i memòria de les rates velles disminuint els nivells d'estrès (Waddell et al. 2010; Yamamoto et al. 2008), tot i que aquests efectes no es van mantenir durant l'aprenentatge de reversió.

La comparació dels resultats obtinguts en les dues tasques ens porta a considerar que la infusió de DCS a l'HPCv ha exercit un efecte facilitador més evident en la TSPA que en el LAM. Una possible explicació podria ser que l'HPCv és una estructura que està més implicada en la tasca de transmissió social que en l'execució del LAM (Ferbinteanu et al. 2003). Cal tenir present que la regió dorsal de l'HPC és la que sembla tenir una major participació en l'aprenentatge espacial (Bannerman et al. 2004), encara que l'HPCv també s'ha relacionat amb la memòria espacial i s'ha mostrat més sensible a la pèrdua de rNMDA associada a l'edat (Loureiro et al. 2012; Liu et al. 2008). D'altra banda, també cal considerar que la DCS es va administrar en una única dosi abans de l'adquisició de la TSPA, mentre que en l'experiment amb el LAM es va injectar durant cinc dies consecutius. S'ha demostrat que l'administració crònica d'aquest agonista del rNMDA pot reduir la seva eficàcia (Quartermain et al. 1994), probablement induint dessensibilització dels receptors i, per tant, podria disminuir el seu potencial per millorar la memòria espacial. Finalment, s'ha observat que la DCS afavoreix el processament de la informació socialment adquirida (Modi & Young 2011), fet que podria explicar

també perquè la DCS va exercir una acció més contundent en la memòria de la TSPA en comparació amb el LAM.

La D-cicloserina actua com un potenciador cognitiu

Els resultats obtinguts corroboren àmpliament la funció que s'ha atorgat als rNMDA en la modulació dels processos cognitius i estan a favor del suggeriment que l'actuació farmacològica en aquests receptors podria contribuir a dissenyar tractaments útils per a millorar les alteracions de memòria presents tant en malalties neurodegeneratives com en el procés normal d'envelliment (Collingridge et al. 2013). La facilitació mitjançant l'administració de DCS dels processos d'extinció i reconsolidació és especialment interessant, ja que suposa avançar en el coneixement i tractament de diferents trastorns relacionats amb la por condicionada, com les fòbies o l'ansietat. No obstant això, cal tenir present que el fet que la DCS pugui induir efectes variables en funció del paradigma i el procediment utilitzat qüestiona la idea del seu paper generalitzat com a potenciador de l'aprenentatge d'extinció i, per tant, és necessari definir sota quines circumstàncies es poden garantir els seus efectes beneficiosos. Quant a l'acció de la DCS com a potenciador de l'adquisició i el record de nous aprenentatges en condicions normals, els nostres resultats indiquen que també sembla dependre del paradigma utilitzat. En aquest treball hem demostrat que permet millorar l'execució de tasques de memòria implícita, com la DSO, però no produeix un efecte notable en tasques de memòria relacional i espacial. L'efecte potenciador de la memòria de la DSO podria ser atribuïble a una millora en la codificació dels estímuls o en la formació inicial de la memòria, ja que l'administració ha estat realitzada abans de l'aprenentatge. El fet de què els seus efectes no s'observin fins a la retenció a les 24 hores, suggereix que possiblement estaria modulant el procés de consolidació primerenca de la memòria. D'altra banda, en rates joves la DCS administrada a diferents regions cerebrals, com són el CPL i l'HPCv, no millora la memòria de la TSPA en el test estàndard de retenció ni quan el procediment comporta major dificultat, així com tampoc quan la memòria es mesura 24 hores o 72 hores després de l'adquisició. Per últim, si bé la DCS no millora l'adquisició de l'aprenentatge espacial, en aquest cas seria convenient provar altres regions cerebrals que participin de manera més rellevant en aquest aprenentatge, com podria ser l'HPCd, així com modificar variables del procediment, com ara el nombre d'assajos, el test de retenció o el protocol d'administració.

D'altra banda, el present treball demostra que la DCS pot ser utilitzada per la reversió dels dèficits de memòria induïts per causes diverses, com ara la disfunció colinèrgica o l'envelliment. Amb tot, les dades resultants de l'experiment 7 són difícils de contrastar amb la literatura, ja que no hi ha estudis previs que hagin investigat els efectes de l'administració intracerebral de DCS en la memòria

dels animals vells. Però si partim de la base que durant l'envelliment sembla existir una alteració de la transmissió colinèrgica i glutamatèrgica, especialment a l'HPC, podríem comparar aquests resultats amb els obtinguts en els experiments 5 i 6, i amb els de Kishi et al. (1998) i Ohno & Watanabe (1996), en els quals es va observar que la DCS injectada a l'HPC era capaç de compensar els dèficits provocats per l'administració d'SCOP en la memòria olfactòria, en la memòria de treball així com en la plasticitat neural. Per tant, podríem suggerir que el mecanisme pel qual la DCS pot recuperar els dèficits cognitius associats a l'edat consisteix a facilitar la plasticitat sinàptica (Rouaud & Billard 2003), la qual es veuria reduïda de manera espontània en animals vells (Barnes 2003).

En resum, els nostres resultats proporcionen proves funcionals que la DCS podria ser un fàrmac d'utilitat per al tractament de dèficits cognitius, probablement mitjançant la millora dels mecanismes de plasticitat sinàptica. Per tant, les dades presents recolzen l'evidència acumulada a favor de l'ús dels agonistes del lloc d'unió de la glicina (Jansen & Dannhardt 2003) com a tractaments potencials per a mitigar l'alteració de la memòria que es manifesta en l'envelliment normal i en les malalties neurodegeneratives.

VI. Conclusions

VI. Conclusions

1. L'administració de DCS, un agonista parcial dels rNMDA, a l'ABL abans de l'entrenament en extinció de la tasca DSO impedeix l'aprenentatge i la retenció a les 24 hores i afavoreix la readquisició posterior de l'aprenentatge original. Aquests efectes podrien ésser atribuïbles a un enfortiment de la traça de memòria original.

2. L'administració de DCS a l'ABL abans de la reactivació de la memòria de la DSO facilita la seva reconsolidació, resultat que dóna suport a la hipòtesi que la DCS administrada preentrenament afavoreix la memòria del condicionament original.

3. La injecció de DCS al CPL abans de l'adquisició de la DSO reverteix els dèficits de memòria provocats per l'administració d'SCOP i facilita el test de retenció realitzat 24 hores després.

4. La infusió de DCS al CPL abans de l'adquisició de la TSPA millora lleugerament els dèficits induïts per l'SCOP quan el test de preferència alimentària a les 24 hores conté dues opcions de resposta, i és capaç de revertir completament el deteriorament quan el test inclou tres alternatives. La DCS per si sola no potencia la memòria de la TSPA en cap dels dos paradigmes.

5. L'ús de DCS directament a l'HPCv permet compensar l'alteració deguda a l'acció de l'SCOP en la memòria de la TSPA avaluada 24 hores després de l'aprenentatge. L'administració només de DCS no potencia la retenció d'aquesta tasca.

6. L'administració de DCS i SCOP no té cap efecte en altres variables que podrien haver intervingut en els resultats, com la capacitat olfactòria, la interacció social, l'activitat motora, la motivació per menjar o els canvis en la neofòbia.

7. La perfusió de DCS en seccions d'HPC és capaç de compensar el deteriorament en el manteniment de la PLT en sinapsis de CA1 promogut per la perfusió d'SCOP. A més, la infusió de DCS per si mateixa amplia els PEP enregistrats abans de la inducció de PLT, tot i que no potencia el seu manteniment.

8. La injecció de DCS a l'HPCv abans de l'adquisició de la TSPA redueix la pèrdua de memòria associada a l'envelliment, ja que produeix una millora en el test de memòria realitzat 72 hores després.

9. L'administració preentrenament de DCS a l'HPCv no millora l'adquisició del LAM ni el test de retenció realitzat 72 hores després, però redueix la rigidesa cognitiva associada amb l'edat i facilita l'aprenentatge de reversió.

VII. Bibliografia

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VIII. Annexes

Annex 1. Manipulacions preentrenament de l'hipocamp ventral

PARADIGMA	MANIPULACIÓ	TEST	RESULTATS	ESTUDI
TASQUES OLFACTÒRIES				
ORDENACIÓ TEMPORAL D'ESTÍMULS	Lesió CA1v	Adq.	↓ estímuls olfactoris = estímuls espacials	Hunsaker i col., 2008
DISCRIMINACIÓ D'OLORS	Lesió NMDA HPCv		↓ aprenentatge	Levita i Muzzio 2012
TASQUES DE POR CONDICIONADA				
POR CONDICIONADA AL CONTEXT	Lesió NMDA HPCv	Adq.	↓ freezing ↑ activitat locomotriu ↓ freezing ↓ defecació en les sessions d'extinció = freezing ↓ preferència pel lloc segur	Richmond i col., 1999 Bannerman i col., 2003 Ferbinteanu i McDonald, 2000 Rudy i Matus-Amat, 2005
	Muscimol HPCv (agonista GABAa)	RT	↓ freezing	
	RU 38486 HPCv (antagonista glucocorticoides)	Adq. RT 24h	= ↓ freezing	Donley i col., 2005
POR CONDICIONADA AL TO I AL CONTEXT	Lesió electrolítica HPCv	Adq.	↓ freezing to i context	Trivedi i Coover, 2004 Maren i Holt, 2004 Maren, 1999
	Lesió electrolítica i neurotòxica SUBv	Adq.	↓ freezing to = freezing context	
		Adq	Lesió CA1v / CA3v: = adq. to i context	
	Lesió àcid ibotènic CA1v i CA3v (agonista NMDA)	RT 48h/72h	Lesió CA1v ↓ freezing context = freezing to	Hunsaker i Kesner, 2008
		RT 24h/48h	Lesió CA3v ↓ freezing context ↓ freezing to	
	Tetrodotoxina HPCv	Adq.	↓ freezing to i context	Bast i col., 2001
	Muscimol HPCv (agonista GABAa)	Adq.	↓ freezing context = freezing to	Maren i Holt, 2004
	Estimulació receptors NMDA HPCv	Adq.	↓ freezing to i context	Zhang i col., 2001
	MK-801 HPCv		↓ freezing context = freezing to	
	Lesió NMDA HPCv	Adq.	↓ freezing context	Czerniawski i col., 2012

	Lesió NMDA <u>HPCv</u>	Adq. Test (1h / 6h / 24h/ 48h /72h)	↓ freezing conext (igual que lesions HPCd)	Wang et al., 2013
POR CONDICIONADA DEMORADA	Lesió NMDA <u>HPCv</u>	Adq.	↓ freezing = amb i sense demora	Yoon i Otto, 2007 Thibaudeau i col., 2007
		Adq. RT 7dies	↓ freezing ↓ freezing	Trivedi i Coover, 2006
	Lesió àcid ibotènic <u>CA1v</u> (agonista NMDA)	Adq. RT 24h RT 48h	= ↓ freezing ↓ freezing	Rogers i col., 2006
	Lesió electrolítica <u>HPCv</u>	Adq. RT 24h	= amb i sense demora = amb i sense demora	Burman i col., 2006
	Muscimol <u>HPCv</u> (agonista GABAc)	Adq.	↓ freezing to i context amb i sense demora	Esclassan i col., 2008
TASQUES D'EVITACIÓ				
EVITACIÓ PASSIVA	Tetrodotoxina <u>HPCv</u> (bloqueig canals Na$^+$)	RT 48h		Lorenzini i col., 1997
	Lesió amb àcid kaïnic <u>CA3v</u> (agonista kainat)	RT 24h/48h/72h/96h	↓	Alvarez i Banzan, 2008
EVITACIÓ ACTIVA	Histamina <u>HPCv</u> (agonista histaminèrgic)	Adq.	↓ ↑ latència de fugida	Alvarez i Banzan, 2001
	Estimulació amb àcid glutàmic <u>HPCv</u>	RT 24h		Alvarez i Ruarte, 2004
	Lesió <u>HPCv</u>	Adq.	Δ	Nadel, 1968
INHIBICIÓ PREPULS (IPP)				
PREPULSE INHIBITION (PPI)	Infusió d'NMDA <u>HPCv</u> (agonista NMDA)	Adq.	↓	Klarner i col., 1998
		Adq.	↓ dosi-dependent ↓ Efecte resistent al pre-tractament amb neurolèptics	Zhang i col., 2002b
		RT 24h	=	Bast i col., 2001b
	Muscimol <u>HPCv</u> (agonista GABAa)	Adq.	Efecte resistent al pre-tractament amb neurolèptics ↓	Zhang i col., 2002a
	Tetrodotoxina <u>HPCv</u> (bloqueig canals Na$^+$) Picrotoxina HPCv (antagonista GABAa)	Adq. RT24h	↓ sobresalt acústic =	Bast i col., 2001c
	Lesió àcid ibotènic <u>HPCv</u> neonatal (agonista NMDA) + Oxotremorina (agonista muscarínic) sistèmica	Adq.	↓ L'administració de biperiden (antagonista muscarínic) bloqueja el dèficit	Laplante i col., 2005
ALTRES PROVES D'ANSIETAT				

Tasca	Manipulació	Fase	Efecte	Referència
LABERINT EN CREU ELEVAT	Lesió àcid ibotènic HPCv (agonista NMDA)	Adq.	↓ ansietat	Kjelstrup i col., 2002
	Lesió citotòxica HPCv			McHugh i col., 2004
	Lesió electrolítica HPCv		↓ evitació passiva = resposta de fugida	Trivedi i Coover, 2004
	Lesió NMDA HPCv	Adq.	↓ ansietat	Bannerman i col., 2002
	Lidocaina 2% HPCv (anestèsic local)			Calfa i col., 2007
	Tetrodotoxina HPCv (bloqueig canals Na^+)	Adq.	↓ ansietat	Degroot i Treit, 2004
	Histamina HPCv (agonista histaminèrgic)	Adq.	↑ ansietat	
	Pyrilamine HPCv (antagonista H_1)	Adq.	↑ ansietat en dosis altes	Rostami i col., 2006
	Ranitidine HPCv (antagonista H_2)		Dosis baixes reverteix efectes de la histamina	
LABERINT EN T ELEVAT	Lesió NMDA HPCv	Adq.	=	Bannerman i col., 2003
CONFRONTACIÓ/INTERACCIÓ SOCIAL	Lesió citotòxica HPCv	Adq.	↓ ansietat	McHugh i col., 2004
	Lesió NMDA HPCv			Bannerman i col., 2002
APROPAMENT AL XOC I *BURYING*	Tetrodotoxina HPCv (bloqueig canals Na^+)	Adq.	= xocs ↓ ansietat	Degroot i Treit, 2004
HIPONEOFÀGIA	Lesió NMDA HPCv	Adq.	↓ ansietat	Bannerman i col., 2003 i 2002
	Lesió neonatal NMDA HPCv		↓ ansietat ↓ interacció social	McHugh i col., 2004
	Lesió citotòxica HPCv		↓ ansietat	McHugh i col., 2004
BLACK/WHITE BOX	Lesió citotòxica HPCv	Adq.	↓ ansietat	Bannerman i col., 2003
	Lesió NMDA HPCv			
EXPOSICIÓ A OLOR DE GAT	Lesió àcid ibotènic HPCv (agonista NMDA)	Adq. RT 24h	↓ ansietat = ansietat	Pentkowski i col., 2006
EXPOSICIÓ A GAT			↓ ansietat	
XOC ELÈCTRIC ALS PEUS			↓ ansietat	
IMMOBILITZACIÓ	Lesió SUBv	Adq.	↑ secreció de glucocorticoides	Herman i col., 1998
TASQUES ESPACIALS				
LABERINT AQUÀTIC	Lesió excitotòxica HPCv	Adq.	↑ execució respecte la lesió d'HPCd	Richmond i col., 1999
			= execució	De Hoz i col., 2003
		Adq. RT	= =	Moser i Moser, 1998

	Lesió NMDA <u>HPCv</u>	Adq.	=	Bannerman i col., 2003
			∇	Ferbinteanu i McDonald, 2000
			↓	Ferbinteanu i col., 2003
	Lesió per aspiració	Adq.	=	Moser i col., 1993
	Lesió citotòxica	Adq.	= ↑ velocitat natació	Bannerman i col., 1999
	Lesió amb àcid kaïnic <u>CA3v</u> (agonista kainat)	Adq.	↓	Stubley-Weatherly i col., 1996
	Galanina HPCv (neuropèptid) 1nmol	Adq. RT 7dies	↑ ↑	
	Galanina HPCv (neuropèptid) 3nmol	Adq. RT 7dies	↓ ↓	Ögren i col., 1996
	Galanina HPCv (neuropèptid) 6nmol	Adq. RT 7dies	= ↓	
LABERINT EN T	Lesió àcid ibotènic <u>HPCv</u> (agonista NMDA)	Adq.	↓ la millora deguda a la nicotina crònica sistèmica	Levin i col., 1999
	Lesió citotòxica <u>HPCv</u>	Adq.	=	Bannerman i col., 1999
	Lesió excitotòxica <u>HPCv</u>	Adq. Adq.	= =	Potvin i col., 2006
	Lesió NMDA <u>HPCv</u>	Adq.	= memòria de treball = memòria de referència	Pothuizen i col., 2004
	Lesió àcid ibotènic <u>HPCv</u> (agonista NMDA)	Adq.	= ↓ la millora deguda a la nicotina crònica sistèmica	Levin i col., 1999
	Lidocaina 2% <u>SUBv</u> (anestèsic local)		↓ win-shift espacial	Black i col., 2004
	Lidocaina 2% <u>HPCv</u> (anestèsic local)	Adq.	↓ memòria de treball ↓ memòria de referència	Poucet i Buhot, 2004
LABERINT RADIAL	Escopolamina <u>HPCv</u> (antagonista muscarínic)		↓	
	Escopolamina <u>i.p.</u> (antagonista muscarínic) + Microdiàlisi per ACh a l'<u>HPCv</u>	Adq.	↓ ↑ alliberació d'ACh ↑ dèficit en la tasca, ↓ Alliberació d'ACh	Mishima i col., 2000
	Escopolamina <u>HPCv</u> (antagonista muscarínic)		↓	
	Pilocarpina <u>HPCv</u> (agonista muscarínic)	Adq.	=	Kim i Levin, 1996

Nicotina <u>HPCv</u> (agonista nicotínic)		=	
Mecamilamina <u>HPCv</u> (antagonista nicotínic)		↓	
MLA <u>HPCv</u> (antagonista α7)	Adq.	↓ dosi-dependent	Bettany i Levin, 2001
		↓	Felix i Levin, 1997
		=	Pocivavsek i col., 2006
	Adq.	↓ memòria de treball = memòria de referència	Levin i col., 2002
	Adq.	↓ memòria de treball ↓ memòria de referència	
DhβE <u>HPCv</u> (antagonista α4β2)		↓	Felix i Levin, 1997
		↓ dosi-dependent	Bancroft i Levin, 2000
	Adq.	↓	Pocivavsek i col., 2006
		↓ a dosis altes	Arthur i Levin, 2002
Dizocilpine <u>HPCv</u> (antagonista NMDA)	Adq.	= ↓ si es coadministra amb nicotina sistèmica	Levin i col., 2003
MK-801 <u>HPCv</u> (antagonsita NMDA)	Adq..	↓ Histamina reverteix dèficits	Xu i col., 2005
Dihydrexina <u>HPCv</u> (agonista D_1)		=	Wilkerson i Levin, 1999
SCH 23390 <u>HPCv</u> (antagonista D_1)		=	
Quinpirole <u>HPCv</u> (agonista D_2)	Adq.	↑ dosi-dependent ↑ millora el dèficit degut a l'escopolamina sistèmica	Fujishiro i col., 2005
Raclopride <u>HPCv</u> (antagonista D_2)	Adq.	↓ dosi-dependent	Wilkerson i Levin, 1999
Quinpirole <u>sistèmic</u> (agonista D_2) + Microdiàlisi per ACh a l'<u>HPCv</u>	Adq.	↓ ↑ alliberació d'ACh a l'HPCv Bloqueig per l'administració d'eticlopride (antagonista D_2)	Umegaki i col., 2001
Microdiàlisi per ACh a l'<u>HPCv</u>	Adq.	Laberint en creu: ↑ alliberació d'ACh	Chang i Gold,

			a l'HPCv	2003
		Adq.	Tasca de lloc: ↑ alliberació d'ACh	Pych i col., 2005
			Tasca de resposta: ↑ alliberació d'ACh	
	Lesió excitotòxica HPCv	Adq.	= tasca espacial i memoria de treball	Jarrard i col., 2012

TASQUES D'APARELLAMENT I NO APARELLAMENT

NO APARELLAMENT DEMORAT AMB LA POSICIÓ	Lesió excitotòxica HPCv	Adq.	=	Potvin i col., 2006
	Muscimol HPCv (agonista GABAa)			Mao i Robinson, 1998
	Escopolamina HPCv (antagonista muscarínic)		↓	Robinson i Mao, 1997
	MK-801 HPCv (antagonista NMDA)		=	
APARELLAMENT DEMORAT AMB LA POSICIÓ AMB I SENSE DEMORA	Lesió NMDA HPCv			Bannerman i col., 2002

TASQUES D'ALTERNANÇA

ALTERNANÇA DEMORADA EN GÀBIA OPERANT	Muscimol HPCv (agonista GABAa)	Adq.	↓	Maruki i col., 2001
ALTERNANÇA ESPONTÀNIA	Lesió HPCv			Stevens i Cowey, 1973
ALTERNANÇA DEMORADA EN LABERINT EN T	Lesió àcid ibotènic HPCv (agonista NMDA)	Lesió neonatal	↓	Lipska i col., 2002
		Lesió adult	=	
ALTERNANÇA REFORÇADA EN LABERINT EN T	Lesió àcid ibotènic SUBv (agonista NMDA)	Adq.	↓	Laxmi i col., 1999
	Lesió NMDA HPCv	Adq.	=	Bannerman i col., 2002

ACTIVITAT LOCOMOTRIU

CAMP OBERT	Lesió NMDA HPCv	Activitat	=	Bannerman i col., 2003
		Activitat	↑	Yoon i Otto, 2007
	Lesió àcid ibotènic HPCv (agonista NMDA) neonatal	Prepuber.	↓ exploració del centre = activitat	Daenen i col, 2001
		Postpuber.	↓ exploració del centre ↓ habituació entorn ↑ distància recorreguda	
	Lesió SUBv	Activitat	↓ exploració del centre ↓ deambulació ↑ responsivitat	Herman i col., 1998
	Tetrodotoxina HPCv (bloqueig canals Na$^+$)	Activitat	↓ dosi-dependent	Bast i col., 2001
	Muscimol HPCv (agonista GABAa)		↓ dosi-dependent	
	Lidocaina 2%		=	Bardgett i

	HPCv (anestèsic local)			Henry, 1999
	Picrotoxina HPCv (antagonista GABAa)		↑ en dosis altes	Bast i col., 2001a
	MK-801 HPCv (antagonista NMDA)		↑	Zhang i col., 2001
			=	
			↓ dosi-dependent	Zhang i col., 2002
	Infusió NMDA HPCv		↑ Hiperactivitat bloquejada per neurolèptics	Bast i col., 2001b
			↑ Hiperactivitat bloquejada per haloperidol, SCH 23390 i reserpina	Bardgett i Henry, 1999
GÀBIA PER MONITORITZAR ACTIVITAT LOCOMOTRIU	Lesió electrolítica HPCv	Activitat	=	Trivedi i Coover, 2004
	Lidocaina 2% HPCv (anestèsic local)			Bardgett i Henry, 1999
	Infusió NMDA HPCv		↑	
ACTIVITAT EN SITUACIONS NOVES	Lesió HPCv	Activitat	↑	Nadel, 1968
ACTIVITAT LOCOMOTRIU INDUÏDA PER AMFETAMINES	Lesió àcid ibotènic HPCv (agonista NMDA) neonatal	Lesió dia 3	↑ postpubertat	
		Lesió dia 14	↑ prepubertat	Wood i col., 1997
ESTEREOTÍPES INDUÏDES PER APOMORFINA		Lesió dia 3	↑	
		Lesió dia 14	↓	

ALTRES TASQUES

HABITUACIÓ	Lesió HPCv		Afectació conducta exploratòria	Nadel, 1968
SENSIBILITZA-CIÓ	Lesió NMDA HPCv		=	Bannerman i col., 2003
ORDENACIÓ TEMPORAL	Lesió CA1v	Adq.	↓ en objectes visuals ↓↓↓ en estímuls olfactoris = en localitzacions espacials	Hunsaker i col., 2008
PHOTOCELL CAGES	Lesió excitotòxica HPCv	Adq.	=	Bannerman i col., 1999
DRL TASK			↓	
APRENENTATGE PROBABILÍSTIC	Lesió HPCv	Adq.	↓	Stevens i Cowey, 1973
GÀBIA OPERANT	Lidocaina 2% SUBv (anestèsic local)	Reinst.	↓	Sun i Rebec, 2003
	Blacofen/Muscimol HPCv (agonistes GABAa)	Reinst. Extinció	↓ =	Rogers i See, 2007
DISCRIMINACIÓ VISUAL	Lesió NMDA HPCv	Adq. Reversal	= ↓	McDonald, 2006
DISCRIMINACIÓ		Adq.	=	

TÀCTIL/ESPACIAL				
PREFERÈNCIA CONDICIONADA AL LLOC	Lesió NMDA HPCv	Adq.	↑	Ferbintea nu i McDonald, 2001
HOT-PLATE TEST (prova de dolor)	Lesió àcid ibotènic HPCv (agonista NMDA) neonatal + Oxotremorina i.p. (agonista muscarínic)	Adq.	↑ latència ↓ sensibilitat al dolor ↑ tremolor, salivació, tª corporal	Laplante i col., 2005
COMPORTAMENT SOCIAL	Lesió àcid ibotènic HPCv (agonista NMDA) neonatal	Adq.	= pre i postpubertat	Daenen i col., 2001
CONDUCTA DE JOC		Adq.	↓ pinning	

Annex 1. Efectes de diferents manipulacions preentrenament en l'hipocamp ventral sobre l'execució de diferents tasques conductuals. [5-CSRTT: tasca de *five-choice serial reaction* time; ∇ i Δ: tendència no significativa]

Annex 2. Manipulacions postentrenament de l'hipocamp ventral

PARADIGMA	MANIPULACIÓ POST-ADQ	TEST	RESULTATS	ESTUDI
TASQUES OLFACTÒRIES				
TRANSMISSIÓ SOCIAL DE PREFERÈNCIA ALIMENTÀRIA	Expressió de c-Fos a CA3v	Immed. 1 dia 2 dies 7 dies	↑ = ↑ =	Smith i col., 2007
	Expressió de c-Fos a GDv	Immed. 1 dia 2 dies 7 dies	↑ = = =	
	Marcatge de c-Fos al SUBv	Immed. 1 dia 2 dies 21 dies	↑ ↑ = =	Ross i Eichenbaum, 2006
	Marcatge de c-Fos a l'HPCv	Immed. 1 dia 2 dies	↑ = ↑	Countryman i col., 2005b
	Marcatge de c-Fos al GDv	Immed. 1 dia 2 dies	↑ = ↑	
	Marcatge de pCREB a l'HPCv	Immed 1 dia 2 dies	= = ↑	
	Marcatge de pCREB al GDv	Immed 1 dia 2 dies	↑ = ↑	
	Marcatge de CREB al GDv	Immed	=	Countryman i Gold, 2007
	Marcatge de CREB a CA3v	Immed	=	
	Marcatge de CREB a CA1v	Immed	=	
	Marcatge de pCREB al GDv	Immed	↑	
	Marcatge de pCREB a CA3v	Immed	↑	
	Marcatge de pCREB a CA1v	Immed	↑	
	Escopolamina HPCv (antagonista M1)	24h	Dèficit parcial	Carballo-Marquez i col., 2009
TASQUES DE POR CONDICIONADA				
POR CONDICIONADA AL CONTEXT	Anisomicina HPCv (inhibidor síntesi proteines)	Immed	1 h 48h	= ↓	Rudy i Matus-Amat, 2005
	Muscimol HPCv (agonista GABAa)	Pre-retenció	=	Hobin i col., 2006
	R165 HPCv (anticos	RT	↓	Fantin i col., 2013

Tasca	Manipulació			Efecte	Referència
	contra nectina 1)				
POR CONDICIONADA A TO I CONTEXT	Muscimol HPCv (agonista GABAa)	Pre-retenció		=	Maren i Holt, 2004
	Lesió electrolítica HPCv	Immed	RT	↓ freezing a to i context	
	Lesió electrolítica i neurotòxica SUBv	Immed	RT	↓ freezing a to i context	Maren, 1999
	Lesió NMDA HPCv	2 dies 12 setmanes	RT	↓ freezing a to i context	Sutherland i col., 2008
POR CONDICIONADA DEMORADA	Lesió NMDA HPCv	24h	7 dies	↓ freezing	Yoon i Otto, 2007
		24h	7 dies	↓ freezing	Trivedi i Coover, 2006
	Lesió electrolítica HPCv	24h	7 dies	=	Burman i col., 2006
TASQUES D'EVITACIÓ					
EVITACIÓ PASSIVA	Tetrodotoxina HPCv (bloqueig canals Na^+)	Immed. 0.25h 1.5h 6h Pre-RT	48h	↓ ↓ = = ↓	Lorenzini i col., 1997
EVITACIÓ ACTIVA	Histamina HPCv	Immed. 15 min	24h	↓ ↓	Alvarez i Banzan, 2008
	Pyrilamine HPCv (antagonista H_1)	Pre-histamina	24h	Bloquegen els efectes de la histamina	
	Ranitidine HPCv (antagonista H_2)		24h		
ALTRES PROVES D'ANSIETAT					
CONFRONTACIÓ/INTERACCIÓ SOCIAL	Expressió de c-Fos a CA1v	1 h		↑	Calfa i col., 2007
	Expressió de c-Fos a CA2v				
	Expressió de c-Fos a CA3v				
	Expressió de c-Fos a GDv				
TASQUES ESPACIALS					
LABERINT AQUÀTIC	Lesió àcid ibotènic HPCv (agonista NMDA)	24h	7 dies	↓	Moser i Moser, 1998
	Muscimol HPCv (agonista GABAa)	Pre-RT	48 h	=	
LABERINT RADIAL NOU CONTEXT	Expressió de c-Fos a l'HPCv	1.5h		↑	Vann i col., 2000
	Expressió de c-Fos a l'HPCv	1.5h			
TASQUES D'ALTERNANÇA					
ALTERNANÇA REFORÇADA EN LABERINT EN T	Lesió àcid ibotènic SUBv (agonista NMDA)	24h	7 dies	=	Laxmi i col., 1999
ACTIVITAT LOCOMOTRIU					
CAMP OBERT	Expressió de c-Fos a CA1v	2h		↑	Hale i col. 2008
	Expressió de c-Fos a SUBv	2h			
ALTRES TASQUES					
GÀBIA OPERANT	Expressió de Fra-1 a l'HPCv	1h		↑	Faure i col., 2006

Annex 2. Efectes de diferents manipulacions postentrenament en l'hipocamp ventral sobre l'execució de diferents tasques conductuals.

Annex 3. Manipulacions preentrenament del còrtex prelímbic

PARADIGMA	MANIPULACIÓ	TEST	RESULTATS	ESTUDI
TASQUES D'EVITACIÓ				
EVITACIÓ PASSIVA	Lesió electrolítica PL	Adq.	= ↑ latència a la reixa electrificada	Jinks i McGregor, 1997
	Lesió àcid quinolínic PL + part del CPFm (agonista NMDA)	Adq.	=	Muir i col., 1996
	SCH23390 (Antagonista D1) / Muscimol / CNQX (Antagonista AMPA) / APV CPFm	Adq. Test 24h	↓	Izquierdo i col., 2007
EVITACIÓ ACTIVA D'UN SENTIT	Lesió electrolítica PL	Adq.	↓	Brito i Brito, 1990
EVITACIÓ ACTIVA DE DOS SENTITS		Adq.	=	Fritts i col., 1998
		Adq.	=	
	Lesió electrolítica CPFm	Adq.	↑ evitacions a l'inici de l'entrenament	Joel i col., 1997
TASQUES DE CONDICIONAMENT CLÀSSIC				
RESPOSTA EMOCIONAL CONDICIONADA	Lesió electrolítica PL+IL	Adq. Extinció Adq.	= ↑ resistència a l'extinció = inhibició condicionada	Morgan i col., 1993
		Extinció Reinst.	= =	Gewirtz i col., 1997
	Lesió electrolítica CPFm	Extinció	=	Garcia i col., 2006
	Lesió 6-OHDA CPFm	Adq. Extinció	= ↑ resistència a l'extinció	Morrow i col., 1999
	Muscimol / APV CPFm	Adq.	↓	Gilmartin i Helmstetter., 2010
	Ifenprodil / Muscimol CPFm	Extinció	↓ Retenció i reaprenentatge	Laurent and Westbrook 2008
	D-cycloserina CPFm (co-agonista glicina)	Extinció	↑	Gupta i col., 2013
CONDICIONAMENT CONTEXTUAL DE LA POR	Registre potencials evocats CPFm	Extinció	↑ ↓ en animals estressats ↑ Administració i.p DCS	Matsumoto 2010
CONDICIONAMENT PEL LLOC	APV CPFm	Adq. Test 48h	↓	Barker and Warburton 2008
AVERSIÓ CONDICIONADA AL GUST	Lesió electrolítica PL	Adq.	↓	Brito i Brito, 1990
	Expressió de c-Fos	Extinció	↑ expressió de c-Fos a PL + IL	Mickley i col., 2005
	D-cycloserina IL	Extinció	↑	Peters i Vries 2013
CONDICIONAMENT PALPEBRAL	Muscimol / APV CPFm	Adq.	↓	Takehara-Nishiuchi i col., 2005
TASQUES DE CONDICIONAMENT INSTRUMENTAL				
GÀBIA OPERANT	Lesió àcid ibotènic PL (agonista NMDA)	Adq.	= ↓ sensibilitat a la	Killcross i Coutureau,

			devaluació del reforç	2003
		Adq.	=	Boulougouris i col., 2007
		Reversal	=	
	Lesió àcid ibotènic PL+IL(agonista NMDA)	Adq.	=	Delatour i Gisquet-Verrier, 1999
			↓ execució dependent de la demora	
	Escopolamina PL (antagon. Muscarínic)	Extinció	=	Maruki i col., 2003
		Extin. 24h	↓	
	Mecamilamina PL (antagon. Nicotínic)	Extinció		
		Extin. 24h	↓	
	Microdiàlisi per ACh al CPFm	Extinció	↑ alliberació d'ACh	Izaki i col., 2001
TASCA DE CONFLICTES CONTEXTUALS (auditiu i visual) DE RESPOSTA	Muscimol PL (agonista GABAa)	Adq.	= execució tasca bimodal = execució assaigs congruents ↓ execució assaigs incrongruents	Marquis i col., 2007

TASQUES DE DISCRIMINACIÓ				
	Lesió mecànica PL	Adq.	=	Li i Shao, 1998
		Reversal	↓	
	Lesió electrolítica PL	Adq.	=	Brito i Brito, 1990
	Lesió NMDA PL (agonista NMDA)	Adq.	=	Chudasama i col., 2001
		Reversal		
	Lesió àcid quinolínic PL+IL (agonista NMDA)	Adq.	= ↓ execució si s'inclou un component de memòria de treball	Ragozzino i col., 2002
	Lesió àcid quinolínic CPFm (agonista NMDA)	Adq.	=	Bussey i col., 1997
DISCRIMINACIÓ VISUAL		Reversal	= ↓ reversal quan els estímuls són difícils de discriminar	
	Sulpiride PL (antagonista D$_2$)	Adq	=	
				Brito i col., 1989
	Escopolamina PL (antagonista muscarínic)	Adq		
		Adq	=	
				Granon i col., 1995
	Bungarotoxina PL (antagonista nicotínic)	Adq		
DISCRIMINACIÓ VISUAL, OLFACTÒRIA I DE TEXTURA	Lesió àcid ibotènic PL+IL (agonista NMDA)	Adq.	= = (intra-modal) ↓ reversal al canviar de pistes visuals a olfactòries i textures i viceversa (extra-modal)	Birrell i Brown, 2000
		Reversal		
DISCRIMINACIÓ OLFACTÒRIA I ESPACIAL	Bupivacaina 2% PL+IL (anestèsic local)	Adq.	= = (intra-modal) ↓ reversal al canviar de pistes olfactòries a espacials i viceversa (extra-modal) ↑ respostes perseveratives	Ragozzino i col., 2003
		Reversal		
DISCRIMINACIÓ VISUO-ESPACIAL	Lesió àcid ibotènic PL+IL (agonista NMDA)	Adq	=	Delatour i Gisquet-Verrier, 1999

DISCRIMINACIÓ ESPACIAL	Escopolamina PL+IL (antagonista muscarínic)	Adq		Ragozzino i Kesner, 1998
TASQUES D'ALTERNANÇA				
ALTERNANÇA DEMORADA EN LABERINT EN T, EN Y I RADIAL	Lesió electrolítica PL	Adq.	↓ execució, que millora amb la pràctica si la demora és curta, però no si és llarga	Brito i Brito, 1990
		Adq.	↓ execució, però són capaços de reaprendre la tasca	Brito i col., 1982
	Lesió 6-OHDA CPFm	Adq.	↓	Bubser i Schmidt, 1990
	SKF 81297 PL (agonista D$_1$)	Adq.	↓ dosi-dependent Millora amb la infusió de SCH 23390 (antagonista D$_1$)	Zahrt i col., 1997
ALTERNANÇA DEMORADA ESPACIAL EN GÀBIA OPERANT	Lesió electrolítica CPFm	Adq.	↓ precisió, però millora amb l'exposició perllongada a les condicions experimentals	Van Haaren i col., 1985
ALTERNANÇA NO DEMORADA EN LABERINT RADIAL	Lesió 6-OHDA CPFm	Adq.	=	Bubser i Schmidt, 1990
TASQUES ESPACIALS				
LABERINT AQUÀTIC	Lesió per radiofreqüència PL+IL	Adq. RT Reversal	= = ↓ execució inicial en reversal intra-modal	De Bruin i col., 1994
	Lesió per radiofreqüència PL	Adq.	↓ execució al canviar de pistes espacials a visuals = des d'1 o 2 punts de sortida diferents ↓ des de 4 posicions de sortida diferents (dèficit específic per les 2 noves posicions introduïdes)	Granon i Poucet, 1995
LABERINT RADIAL	Lesió electrolítica PL	Adq.	↓	Fritts i col., 1998
		Adq. Reversal	= =	Joel i col., 1997
	Lesió àcid ibotènic PL (agonista NMDA)	Adq.	= ↓ si la localització diana és variables	Delatour i Gisquet-Verrier, 1996
	Lesió àcid ibotènic PL+IL (agonista NMDA)	Adq.	Estratègia *win-shift* no demorada: = ↓ transitòria a l'introduir 1' de demora	Delatour i Gisquet-Verrier, 2006
			Estratègia *win-shift* demorada: = sense impediment a l'augmentar la demora de 5' a 30' ↓ a l'introduir un element distractor durant la demora	
	Lesió excitotòxica PL+AC	Adq.	*win-shift* demorat: ↑ errors inter- i intra-fase ↑ latència	Taylor i col., 2003
	Lesió àcid quinolínic PL+IL	Adq.	↓ discriminació espacial	Ragozzino i col.,

	(agonista NMDA)		transitòria ↓ memòria de treball espacial	1998
	Lidocaina 2% PL (anestèsic local)	Adq.	Estratègia *win-shift* demorada: = ↓ memòria de treball Estratègia *win-shift* no demorada: = ↓ execució al canviar d'un paradigma espacial a un de cerca de menjar	Seamans i col., 1995
	Escopolamina PL+IL (antagonista muscarínic)	Adq.	↓ dosi-dependent Millora amb l'administració d'oxotremorina concomitant	Ragozzino i Kesner, 1998
	SCH 23390 PL (antagonista D_1)	Adq.	Estratègia *win-shift* demorada: ↓ memòria de treball dosi-dependent Estratègia *win-shift* no demorada: =	Seamans i col., 1998
	Sulpiride PL (antagonista D_2)	Adq.	Estratègia *win-shift* demorada: = memòria de treball Estratègia *win-shift* no demorada: =	
	Estradiol PL	Adq.	↑ memòria de treball	Sinopoli i col., 2006
	MK-801 CPFm (antagonista NMDA)	Adq. Test 1h	↓ memòria de treball	Rios-Valentin i col., 2009
LABERINT EN CREU	Muscimol PL+IL (agonista GABAa)	Adq. Reversal	= = canvi d'estratègia = reversió espacial	Rich i Shapiro, 2007
	Tetracaina 2% PL+IL (anestèsic local)	Adq. Reversal	Discriminació de lloc i resposta: = adquisició de lloc i resposta = reversió intra-modal ↓ reversió cross-modal	Ragozzino i col., 1999a
	SCH 23390 PL+IL (antagonista D_1)	Adq. Reversal	Discriminació visual i de resposta: = adquisició visual i resposta ↓ reversió cross-modal ↑ respostes perseveratives	Ragozzino, 2002
	MK-801 CPFm (antagonista NMDA)	Reversal	↓	Watson and Stanton 2009
TASCA ESPACIAL *CHEESE-BOARD*	Tetracaina 2% PL+IL (anestèsic local)	Adq. Reversal	Discriminació visual i espacial = adquisició visual i espacial ↓ reversió cross-modal	Ragozzino i col., 1999b
TASCA ESPACIAL DE LOCALITZACIÓ D'UNA CAIXA ENTRE 13	Lesió àcid ibotènic PL+IL (agonista NMDA)	Adq.	= des d'una posició de sortida ↓ des de 4 posicions de sortida	Delatour i Gisquet-Verrier, 2000

TASQUES D'APARELLAMENT I NO APARELLAMENT				
NO APARELLAMENT DEMORAT AMB LA MOSTRA	Lesió electrolítica CPFm	Adq. Reversal	= ↓ reversió d'un paradigma d'aparellament a un de no aparellament	Joel i col., 1997b
	Lesió per radiofreqüència PL	Adq.	↓	Granon i col., 1994
	Escopolamina PL (antagonista muscarínic)	Adq.	↓	Brito i col., 1989
		Adq.	↓	Granon i col., 1995
	Bungarotoxina PL (antagonista nicotínic)	Adq.	=	
	Sulpiride PL (antagonista D_2)	Adq.	=	Brito i col., 1989
APARELLAMENT DEMORAT AMB LA MOSTRA	Lesió per radiofreqüència PL	Adq.	↓	Granon i col., 1994
	Escopolamina PL (antagonista muscarínic)			Granon i col., 1995
	Bungarotoxina PL (antagonista nicotínic)			
NO APARELLAMENT DEMORAT AMB LA POSICIÓ	Lesió àcid ibotènic PL+IL (agonista NMDA)	Adq.	= ↓ si la demora és variable, però no si és fixa = = encara que s'augmenti la demora	Delatour i Gisquet-Verrier, 1996; 2000 Gisquet-Verrier i col., 2000
	Lesió NMDA PL+IL (agonista NMDA)	Adq.	= ↓ si la lesió inclou l'AC	Dias i Aggleton, 2000
	Lesió NMDA CPFm (agonista NMDA)	Adq.	↓ precisió ↑ biaix	Aggleton i col., 1995
	Lesió NMDA PL (agonista NMDA)	Adq.	Dèficits dependents i no dependents de la demora	Chudasama i Muir, 1997
	Escopolamina PL (antagonista muscarínic)		↓ precisió ↑ latència de resposta	
APARELLAMENT DEMORAT AMB LA POSICIÓ	Lesió NMDA PL+IL (agonista NMDA)	Adq. Reversal	↓ adq. ↑ perseverança ↓ reversió d'aparellament a no aparellament	Dias i Aggleton, 2000
	Escopolamina CPFm (antagonista muscarínic)	Adq.	↓ dependent de la dosi i de la demora	Broersen i col., 1994
	Escopolamina PL (antagonista muscarínic)	Adq.	↓ dependent de la dosi i de la demora ↑ latència de resposta	Broersen i col., 1995
	Apomorfina PL (antagonista DA)	Adq.	=	
	Apomorfina CFPm (antagonista DA)		↑ latència de resposta ↓ freqüència de *nose-pokes* dosi-dependents	Broersen i col., 1994
	Cis-flupenthixol CPFm (antagonista DA)			
	Cis-flupenthixol PL (antagonista DA)	Adq..	↓ dependent de la dosi i de la demora ↑ latència de resposta ↓ freqüència de *nose-pokes*	Broersen i col., 1995
	SCH 23390 PL (antagonista D_1)			
TASQUES ATENCIONALS				
5-CSRTT	Lesió àcid ibotènic PL+IL (agonista NMDA)	Adq.	↓ precisió inicial ↑ respostes perseveratives dependent de la demora	Passetti i col., 2002
		Adq.	↓ precisió ↑ respostes perseveratives i errors d'omissió	Passetti i col., 2003

	Lesió àcid quinolínic PL+CPFm (agonista NMDA)	Adq.	↓ precisió ↑ latència de resposta ↑ respostes perseveratives	Muir i col., 1996
	Lesió amb 192 IgG-Saporina PL+IL (neurotoxina colinèrgica selectiva)	Adq.	↓ vigilància ↓ precisió quan les demandes atencionals són elevades ↑ respostes perseveratives quan els EE es presenten imprevisiblement ↑ errors d'anticipació	Dalley i col., 2004b
	Sulpiride PL (antagonista D$_2$)	Adq.	=	Granon i col., 2000
	SCH 23390 PL (antagonista D$_1$)	Adq.	↓ precisió en subjectes amb nivells basals alts	
	SKF 38393 PL (agonista D$_1$)	Adq.	↑ precisió en subjectes amb nivells basals baixos Els efectes es bloquegen amb la infusió d'SCH 23390	
	Nicotina PL (agonista nicotínic)	Adq.	↑ precisió dosi-dependent ↑ errors d'omissió a l'inici	Hahn i col., 2003
	Microdiàlisi per ACh i NA al CPF	Adq..	↑ alliberació d'ACh i NA	Dalley i col., 2001
	Microdiàlisi per ACh al CPFm		↑ alliberació d'ACh	Passetti i col., 2000
	Lesió amb 192 IgG-Saporina NBM (neurotoxina colinèrgica selectiva) + Microdiàlisi per ACh al CPFm	Adq.	↓ funció atencional ↑ alliberació d'ACh a l'inici de la tasca ↓ alliberació d'ACh durant la tasca	McGaughy i col., 2002
	CPP CPFm	Adq. Test	↓ funció atencional	Murphy i col., 2005
	APV CPFm (antagonista NMDA)	Adq.	↓ funció atencional Model Esquizofrènia	Carli i col., 2011
	CPP CPFm	Adq,	↓ funció atencional ↑ Impulsivitat	Pozzi i col., 2011
3-CSRTT	Lesió per radiofreqüència CPFm	Adq.	↓ respostes correctes ↑ errors d'omissió ↓ latència de resposta	Broersen i Uylings, 1999
TASCA VISUAL D'ATENCIÓ SOSTINGUDA	Lesió amb 192 IgG-Saporina NBM (neurotoxina colinèrgica selectiva) + Registre electrofisiològic al CPFm	Adq.	↓ funció atencional ↓ activitat neuronal general ↑ activitat neural quan augmenta la demanda atencional	Gill i col., 2000
	APV al PB (antagonista NMDA) + Microdiàlisi per ACh al CPFm	Adq.	↓ atenció sostinguda ↓ habilitat per detectar les senyals ↑ alliberació d'ACh correlacionant amb l'augment de la demanda atencional	Kozak i col., 2006

Tasca	Manipulació	Fase	Efecte	Referència
CAM (tasca combinada d'atenció i memòria)	Escopolamina PL+AC (antagonista muscarínic)	Fase atencional	= precisió ↑ errors d'omissió ↑ latència de resposta ↑ respostes prematures dosi-dependent	Chudasama i col., 2004
		Fase de memòria: Immed 4'' 8'' 16''	↓ precisió dosi-dependent i independent de la demora ↑ latència de resposta	
VISUAL TIMING TASK (atenció sostinguda i inhibició de resposta)		Adq.	↓ respostes correctes ↑ respostes prematures ↑ errors d'omissió	Broersen i Uylings, 1999
TASCA VISUAL D'ATENCIÓ SELECTIVA	Lesió per radiofreqüència CPFm	Adq.	= quan les demandes atencionals són baixes (2 estímuls)	Granon i col., 1998
TASCA VISUAL D'ATENCIÓ SOSTINGUDA		Adq.	↓ atenció sostinguda per detectar i reaccionar a variacions subtils de brillantor	
TASCA DE DETECCIÓ DE SENYAL	Escopolamina PL (antagonista muscarínic)	Adq.	↓ precisió quan la senyal apareix de forma aleatòria = precisió quan la senyal apareix de forma predictiva	Williams i col., 1999
SET-SHIFTING ATENCIONAL	Benoxathian CPFm (antagonista α1-adrenèrgic)	Adq.	Bloqueig de la millora de l'atipamezole (antagonista auto-recep. α2-adrenèrgic) sistèmic	Lapiz i Morilak, 2006
	Betaxolol CPFm (antagonista β-adrenèrgic)	Adq.	No bloqueja la millora	
TASQUES MOTORES				
DOLOR NEUROPÀTIC	DCS PL	Adq.	↓ Conducta de dolor	Millecamps i col., 2007
TASQUES OLFACTÒRIES				
TRANSMISSIÓ SOCIAL DE PREFERÈNCIA ALIMENTÀRIA	Escopolamina PL (antagonista muscarínic)	RT Immed RT 24h	↓ ↓	Boix-Trelis i col., 2007
DISCRIMINACIÓ SIMPLE D'OLORS	Registre electrofisiològic PL		↑ activitat neuronal durant l'aprenentatge de la tasca	Kublik i Sara, 2002
	D-cycloserine PL (co-agonista glicina)	Adq. Test 24h	↑ Retenció	Villarejo-Rodriguez i col., 2011
	D-cycloserine PL (co-agonista glicina) + Lesió PF	Adq. Test 24h	↑ Reversió dèficits deguts a la lesió	Villarejo-Rodriguez i col., 2012
INFERÈNCIA TRANSITIVA	Lesions PL-IL		↓	DeVito i col., 2010

Annex 3. Efectes de diferents manipulacions preentrenament en el còrtex prelímbic sobre l'execució de diferents tasques conductuals. [6-OHDA: 6-hidroxidopamina; 5-CSRTT: tasca de *five-choice serial reaction* time; 3-CSRTT: tasca de *three-choice serial reaction time*]

Annex 4. Manipulacions postentrenament del còrtex prelímbic

PARADIGMA	MANIPULACIÓ	TEST	RESULTATS	ESTUDI
TASQUES ESPACIALS				
LABERINT EN CREU	Muscimol PL+IL (agonista GABAa)	24h	↓ retenció de l'estratègia apresa 24 hores abans	Rich i Shapiro, 2007
TASQUES D'APARELLAMENT I NO APARELLAMENT				
NO APARELLAMENT DEMORAT AMB LA MOSTRA	Lesió per radiofreqüència PL	7dies	↓ dèficits inicials, però recupera el nivell d'execució normal després de la lesió	Granon i col., 1994
APARELLAMENT DEMORAT AMB LA MOSTRA			↓ encara que tingui experiència prèvia amb la tasca prelesió, mostra dèficits	
TASQUES OLFACTÒRIES				
DISCRIMINACIÓ SIMPLE D'OLORS	APV PL (antagonista NMDA)	48h	↓	Tronel i Sara, 2003
	Timolol PL (antagonista. β-adrenèrgics)	48h	= administració 5 min post ↓ administració 2h post	Tronel i col., 2004
	Microdiàlisi per NA al PL	32m	↑	
		48m	=	
		64m	=	
		80m	=	
		96m	=	
		112m	=	
		128m	↑	
		144m	=	
	Expressió de c-Fos al PL	1.5h	↑	Tronel i Sara, 2002
	Escopolamina (antagonista muscarínic)	24h	↓ Retenció i reaprenentatge	Carballo-Marquez i col., 2008
TASQUES DE RECONEIXEMENT				
RECONEIXEMENT OBJECTES	APV CPFvm (antagonista NMDA)	24h 3h	↓ Retenció i reconsolidació =	Akirav and Maroun 2006

Annex 4. Efectes de diferents manipulacions postentrenament en el còrtex prelímbic sobre l'execució de diferents tasques conductuals.

Annex 5. Manipulacions preentrenament de l'amígdala basolateral

PARADIGMA	MANIPULACIÓ	TEST	RESULTATS	ESTUDI
TASQUES D'EVITACIÓ				
EVITACIÓ INHIBITÒRIA	Lesió BL	Adq.	=	Roozendaal i McGaugh, 1997a
	Lesió àcid ibotènic BL (agonista NMDA)	RT 48h	=	Tomaz i col., 1992
	Lesió NMDA BL (agonista NMDA)	RT 48h	=	Malin i col., 2007
		RT 24h	↓ dosi-dependent	Liang i col., 1994 Roesler i col., 2000a
	APV BL (antagon. NMDA)	RT 24h	↓	Roesler i col., 2000b
		RT 48h	↓	LaLumiere i col., 2004b
	APV BL+Ce (antagon. NMDA)	RT 1.5h	=	Bianchin i col.,

	CNQX <u>BL+Ce</u> (antagon. AMPA/Kainat)	RT 24h	↓	1999
	NA <u>BL+Ce</u> (agonista NA)	RT 1.5h RT 24h	= ↑	
	Escopolamina <u>BL+Ce</u> (antagon. Muscarínic) Escopolamina <u>BL</u> (antagon. Muscarínic)	RT 1.5h RT 24h	= ↓	Barros i col., 2002
	Oxotremorina <u>BL</u> (agonista muscarínic)	RT 1.5h RT 24h	= ↑	
	Mecamilamina <u>BL</u> (antagon. Nicotínic)	RT 1.5h RT 24h	↓ ↓	Barros i col., 2005
	Nicotina <u>BL</u> (agonista nicotínic)	RT 1.5h RT 24h	↑ ↑	
	Atropina <u>BL</u> (antagon. ACh)	RT 48h	No bloqueja la millora deguda a la infusió de RU 28362 a l'HPCd ni ipsi ni contralateral	Roozendaal i col., 1999
	Atenolol <u>BL</u> (antagon. β-adrenèrgics)	RT 48h	Bloqueja la millora deguda a la infusió de RU 28362 a l'HPCd ipsilateral	
	Infusió benzodiacepines (agonista recep. GABA) i.p.	Adquisició Retenció 48h	= ↓	Tomaz i col., 1992
	Lesió BL + benzodiacepines i.p.	Adquisició Retenció 48h	= =	
	Picrotoxina <u>BL +Ce</u> (antagon. GABAa)	RT 1.5h RT 24h	= ↑	Bianchin i col., 1999
	Staurosporin <u>BL +Ce</u> (inhibidor PKC) Kn-62 <u>BL +Ce</u> (inhibidor PK II)	RT 1.5h RT 24h	= ↓	
EVITACIÓ PASSIVA	Atropina <u>BL</u> (antagon. muscarínic)	Adq.	↓	Duméry i Blozovski, 1987
	Arecolina <u>BL</u> (agonista muscarínic)	Adq.	= Bloqueja el dèficit de l'atropina	
	Mecamilamina <u>BL</u> (antagon. Nicotínic)	Adq.	↓ dosi-dependent	Blozovski i Duméry, 1987
	Nicotina <u>BL</u> (agonista nicotínic)	Adq.	↑ lleu Bloqueja el dèficit de la mecamilamina	
EVITACIÓ ACTIVA DE DOS SENTITS	APV <u>BL</u> (antagon. NMDA) + Expressió de c-Fos	Adq RT 48h	↓ ↓ ↓ expressió de c-Fos	Savonenko i col., 2003

TASQUES DE POR CONDICIONADA				
CONDICIONAMENT CLÀSSIC DE POR		RT 24h	↓	Vazdarjanova i McGaugh, 1998
	Lesió NMDA <u>BL</u> (agonista NMDA)	Adq.	↓	Maren i col., 1996a
		Adq.	= efecte del sobreaprenentatge	Maren i col., 1998
		Adq.	Enlentiment de l'adquisició	Maren, 1999
	Lesió electrolítica <u>BL</u>	Adq. Adq.	↓ resposta d'ensurt	Campeau i Davis, 1995
	APV <u>BL</u> (antagon. NMDA)	Adq. RT	↓ ↓	Maren i col., 1996b
		Adq.	↓	Fanselow i Kim, 1994

		Adq.	↓ (to i context)	Goosens i Maren, 2003
	NBQX <u>BL</u> (antagon. AMPA/Kainat)	Adq.	↓ condic. a la olor = to i context	Walker i col. 2005
		Adq.	=	
		Adq. RT	↓ to i context ↓ to i context	Muller i col., 1997
		Adq.	↓	Wilensky i col., 1999
	Muscimol <u>BL</u> (agonista GABAa)	Adq.	↓ dosi-dependent	Wilensky i col., 2000
		Adq. RT 24h	↓ ↓	Helmstetter i Bellgowan, 1994
		Adq.	↓	Maren i col., 2001
		RT 24h	↓	Takahashi i col., 2007
	Anisomycin <u>BL</u>	RT 24h RT 20h	↓ reconsolidació	Nader i col., 2000
	Citotoxin Phalloidin <u>BL</u> (inhibidor filaments actina)	RT 24h	↓ reconsolidació ↓ consolidació	Rehberg i col., 2010
EXTINCIÓ POR CONDICIONADA	Muscimol <u>BL</u> (agonista GABAa)	Adq.	Enlentiment de l'adquisició	Ponnusamy i col., 2007
	D-cycloserina <u>BL</u> (co-agonista glicina)	RT 24h	↑ reconsolidació ↑ extinció	Lee i col., 2006 Walker i col., 2002
TASQUES OLFACTÒRIES I GUSTATIVES				
		Adq.	↑	Schoenbaum i col., 1998
	Registre electrofisiològic del nucli <u>BL</u>	Adq. Reversal	↑ ↑	Schoenbaum i col., 1999
DISCRIMINACIÓ OLFACTÒRIA		Adq. Reversal	↑ go/no-go ↑ go/no-go	Schoenbaum i col., 2000
	Lesió NMDA <u>BL</u> (agonista NMDA)	Adq. Reversal RT	= = ↓ RT del reversal	Schoenbaum i col., 2003
	Lesió àcid ibotènic <u>BL</u> (agonista NMDA)	Adq.	=	Hatfield i col., 1992
TRANSMISSIÓ SOCIAL DE PREFERÈNCIA ALIMENTÀRIA	Lesió àcid ibotènic <u>BL</u> (agonista NMDA)	RT Immed RT 24h	= =	Burton i col., 2000
	Muscimol <u>BL</u> (agonista GABAa)	RT 24h RT 7d	↓ ↓	Wang i col., 2006
	Escopolamina <u>BL</u> (antagonista M1)	RT 24h	↓ adquisició	Carballo-Marquez i col., 2008
	Lesió <u>BL</u>	Adq.	↑ consum aliment nou	
	NA <u>BL</u> (agonista NA)	Adq.	↑ consum aliment familiar	
PREFERÈNCIA ALIMENTÀRIA	Lesió 6-OHDA <u>BL</u> (depleció NA)	Adq.	=	Borsini i Rolls, 1984
	5-HT <u>BL</u> (agonista 5-HT)	Adq.	=	
	Lesió 5,7 dihidroxitriptamina <u>BL</u> (depleació 5-HT)	Adq.	=	
	Lesió electrolítica <u>BL</u>	Adq.	↓	Bermúdez-Rattoni i col., 1986
	d-APV <u>BL</u> (antagon. NMDA)	Adq.	↓ dosi-dependent	Hatfield i Gallagher, 1995
AVERSIÓ CONDICIONADA A UNA OLOR	APV <u>BL</u> (antagon. NMDA)	Adq.	↓	
	NBQX <u>BL</u> (antagon. AMPA/Kainat)	Adq.	=	Walker i col., 2005
	Propanolol <u>BL</u> (antagon. β-adrenèrgics)	Adq.	↓	Miranda i col., 2007
	Bicuculline <u>BL</u> (antagon. GABA)	Adq.	↑	Ferry i Di Scala, 1997

PREFERÈNCIA OLFACTÒRIA TEST DE PERCEPCIÓ OLFACTÒRIA	Lesió àcid ibotènic BL (agonista NMDA)	Adq.	=	Touzani i Sclafani, 2005
		Adq.	=	Ferry i col., 1995
AVERSIÓ CONDICIONADA AL GUST		Adq.	↓	Morris i col., 1999
	Lesió àcid ibotènic BL (agonista NMDA)	Adq.	=	Hatfield i col., 1992
		Adq.	↓	Yamamoto i Fujimoto, 1991
	Muscimol BL (agonista GABAa)	Adq.	=	Ferry i col., 1995
		Adq.	=	
		Adq.	↓	Wang i col., 2006
		Adq.	↓	Bermúdez-Rattoni i col., 1986
	Lesió electrolítica BL	Adq.	↓	Simbayi i col., 1986
		Adq.	↓	Rollins i col., 2001
	NA BL (agonista NA)	Adq.	↓	
		Adq.	=	Borsini i Rolls, 1984
	Lesió 6-OHDA BL (depleció NA)	Adq.	↓	
	Clenbuterol BL (agonista β-adrenèrgics)	Adq.	=	Miranda i col., 2003
	Propanolol BL (antagon. β-adrenèrgics)	Adq.	↓	
		Adq.	↓ apr. accidental = condicionament	Miranda i col., 2008
	5-HT BL (agonista 5-HT)	Adq.	=	Borsini i Rolls, 1984
	Lesió 5,7 dihidroxitriptamina BL (depleció 5-HT)	Adq.	=	
	Microdiàlisi per Glutamat	Adq.	↑ alliberació quan s'administra l'estímul incondicionat (LiCl)	Miranda i col., 2002
POTENCIACIÓ DE L'AVERSIÓ OLFACTÒRIA PER L'ASSOCIACIÓ AMB EL GUST	Lesió àcid ibotènic BL (agonista NMDA)	Adq.	↓	Hatfield i col., 1992
		Adq.	↓	Ferry i col., 1995
	Lesió electrolítica BL	Adq.	↓	Bermúdez-Rattoni i col., 1986
	Muscimol BL (agonista GABAa)	Adq.	↓	Ferry i col., 1995
	d-APV BL (antagon. NMDA)	Adq.	↓	Ferry i Di Scala, 2000
ALTRES TASQUES ASSOCIATIVES				
PREFERÈNCIA A UN LLOC CONDICIONADA	Bupivacaina BL (anestèsic local)	Adq.	↓	Hsu i col., 2002
	Lesió NMDA BL (agonista NMDA)	Adq.	↓	Fuchs i col., 2002
		Adq.	↓	
CONDICIONAMENT INSTRUMENTAL REFORÇAT AMB DROGA		Reinst.	↓	Meil i See, 1997
	Lidocaina 2% BL (anestèsic local)	Reinst.	↓	Kantak i col., 2002
		Reinst.	↓	McLaughlin i See, 2003
	Tetrodotoxina BL (bloqueig canals Na$^+$)	Reinst.	↓	Fuchs i See, 2002
		Reinst.	↓	
		Reinst.	↓ extinció un cop reinstaurada	Fuchs i col., 2006
	Escopolamina BL (antagon. Muscarínic)	Adq.	↓	See i col., 2003
		Reinst.	=	
	APV BL (antagon. NMDA)	Reinst.	↓	
		Reinst.	↓ extinció un cop reinstaurada	Feltenstein i See, 2007
		Reinst.	=	See i col., 2001

	CNQX BL (antagon. AMPA/Kainat)	Reinst.	=	
	SCH 23390 BL (antagon. D$_1$)	Reinst.	↓	
		Reinst.	↓	Berglind i col., 2006
	Raclopride BL (antagon. D$_2$)	Reinst.	↑ dosis baixes ↓dosis altes	
		Reinst.	=	See i col., 2001
	SCH 23390 + Raclopride	Reinst.	↓	
	D-amfetamina BL (facilitació monoaminèrgica)	Pre-extin Reinst.	= extinció ↑	Ledford i col., 2003
	Anisomycion BL	RT 24h	↓ reconsolidació	Fuchs i col., 2009
	Inactivació BL	Adq. Extin	↑ extinció ↓ extinció	Sun i Laviolette 2012
TASCA DISCRIMINATIVA	APV BL (antagon. NMDA)	Adq.	↓ dosi-dependent	Burns i col., 1994
TASQUES DE MEMÒRIA DE TREBALL				
LABERINT AQUÀTIC	Lesió BL	Adq. RT 48h	= =	Roozendaal i McGaugh, 1997a
	RU 38486 BL (antagon. Glucocorticoides)	Adq. RT 48h	= ↓	
		Adq. RT 48h	=	Roozendaal i McGaugh, 1997b
	APV BL (antagon. NMDA)	Adq.	=	Liang i col., 1994
NO APARELLAMENT DEMORAT AMB LA MOSTRA	Lesió neurotòxica BL	Adq.	= ↑ respostes neofòbiques	Aggleton i col., 1989
	Lesió electrolítica BL	Adq.	↓ quan els estímuls són visuals o tàctils = pistes espacials	Peinado-Manzano, 1990
TASCA ESPACIAL I NO ESPACIAL	Lesió BL	Adq.	=	Wan i col., 1994
EVITACIÓ INHIBITÒRIA	Oxotremorina BL (agonista muscarínic)	Adq.	↑ memòria de treball	Barros i col., 2002
	Escopolamina BL (antagon. Muscarínic)	Adq.	↓ memòria de treball	
		Adq.	= memòria de treball	Bianchin i col., 1999
	Nicotina BL (agonista nicotínic)	Adq.	↑ memòria de treball	
	Mecamilamina BL (antagon. Nicotínic)	Adq.	↓ memòria de treball	Barros i col., 2002
	CNQX BL (antagon. AMPA/Kainat)	Adq.	= memòria de treball	
	APV BL (antagon. NMDA)	Adq.	= memòria de treball	
	NA BL (agonista NA)	Adq.	= memòria de treball	
	Picrotoxina BL (antagon. GABAa)	Adq.	= memòria de treball	Bianchin i col., 1999
	Staurosporin BL (inhibidor PKC)	Adq.	= memòria de treball	
	Kn-62 BL (inhibidor PKII dependent de Calci)	Adq.	= memòria de treball	
ALTERNANÇA DEMORADA EN LAB. EN T	Lesió NMDA BL (agonista NMDA)	Adq.	=	Roozendaal i col., 2004
THREE-PANEL RUNWAY TASK	CPP BL (antagon.NMDA)	Adq.	↑ errors en el test de memòria de treball = en memòria de referència	Ohno i col., 1993
	Escopolamina BL (antagon. Muscarínic)	Adq.	↑ errors en el test de memòria de treball = en memòria de referència	

ALTRES TASQUES				
LABERINT ELEVAT EN T	Lidocaina 2% BL (anestèsic local)	Adq.	↓	Tomaz i col., 2003
CAMP OBERT	Lesió NMDA BL (agonista NMDA)	Adq.	= activitat locomotriu	Gale i col., 2004

Annex 5. Efectes de diferents tractaments experimentals preentrenament en l'amígdala basolateral sobre l'adquisició i la retenció de diverses tasques.

Annex6. Manipulacions postentrenament de l'amígdala basolateral

PARADIGMA	MANIPULACIÓ POST-ADQ	TEST	RESULTATS	ESTUDI
TASQUES D'EVITACIÓ				
EVITACIÓ INHIBITÒRIA	Clenbuterol BL (agonista β-adrenèrgics)	Immed	48 h	1.0 ng: = 10.0 ng: ↑ 100.0 ng: = 1000.0 ng: =	Ferry i McGaugh, 1999
		Immed	48 h	↑ dosi-dependent	Ferry i col., 1999a
		Immed	48 h	↑ La millora es bloqueja per la infusió prèvia d'RU 38486	Roozendaal i col., 2002
		Immed	48 h	↑ BL dreta = BL esquerra	LaLumiere i McGaugh, 2005
		Immed	48 h	↑ dosi-dependent	Roozendaal i col., 2008a
	Propanolol BL (antagon. β-adrenèrgics)	Immed	48 h	Bloqueja la millora deguda a la infusió de DA	LaLumiere i col., 2004a
		Immed 3h	48 h	Potencia el dèficit degut a la infusió de OFQ/N	Roozendaal i col., 2007
	Atenolol BL (antagon. β-adrenèrgics)	Immed	48 h	Bloqueja la millora deguda a la infusió de [Nphe^1]nociceptin(1-13)NH$_2$	
		Immed	48 h	Bloqueja la millora deguda a la infusió CRF$_{6-33}$	Roozendaal i col., 2008a
	Cirazoline BL (agonista α-adrenèrgic)	Immed	48 h	↑	
		Immed	48 h	=	
	Prazosin BL (antagon. α-adrenèrgic)	Immed	48 h	Atenua la millora deguda al clenbuterol	Ferry i col., 1999a
		Immed	48 h	No afecta a la millora deguda al 8-bromo-AMPc	
	Phenylephrine BL (agonista α-adrenèrgic)	Immed	48 h	↓ dosi-dependent	
		Immed	48 h	↓ dosis baixes = dosis altes	Ferry i col., 1999b
	Yohimbine BL (antagon. α-adrenèrgic)	Immed	48 h	↑ dosi-dependent	
	APV BL (antagon. NMDA)	Immed Pre-RT	24h	↓ =	Roesler i col., 2000b

	Immed	48h	↑ xoc fort / ↓ xoc fluix	LaLumiere i col., 2004b
	6h		=	
AP3 <u>BL</u> (antagon. Recep. metabotròpics tipus I glutamat)	Immed		↓	
	0.5h		↓	
	1.5h	24h	=	
	3h		=	
	4.5h		=	
	6h		=	Bonini i col., 2003
CNQX <u>BL</u> (antagon. AMPA/Kainat)	Immed		↓	
	0.5h		↓	
	1.5h	24h	↓	
	3h		↓	
	4.5h		=	
	6h		=	
Atropina <u>BL</u> (antagon. ACh)	Immed	48h	Bloqueja la millora deguda a la infusió de DA	LaLumiere i col., 2004a
	Immed	48h	↑ BL dreta / = BL esquerra	LaLumiere i McGaugh, 2005
Oxotremorina <u>BL</u> (agonista muscarínic)	Immed	48h	↑	Malin i col., 2007
	Immed	1.5h	=	Barros i col., 2002
		24h	↑	
	Immed	48h	↑	
+ Telenzipine (agonista M$_1$) + Methoctramine (antagon. M$_2$) + Telenzipine i Methoctramine	Immed	48h	Els antagonistes M$_1$ i M$_2$ bloquegen la millora deguda a l'oxotremorina	Power i col., 2003a
Escopolamina <u>BL</u> (antagon. Muscarínic)	Immed	1.5h	=	Barros i col., 2002
		24h	↓	
Mecamilamina <u>BL</u> (antagon. Nicotínic)	Immed	1.5h	↓	
		24h	↓	Barros i col., 2005
Nicotina <u>BL</u> (agonista nicotínic)	Immed	1.5h	↑	
		24h	↑	
SCH 23390 <u>BL</u> (antagon. D$_1$)	Immed	48h	↓	
Sulpiride <u>BL</u> (antagon. D$_2$)	Immed	48h	↓	LaLumiere i col., 2004a
DA <u>BL</u> (agonista DA)	Immed	48h	↑	
	3h		=	
	Immed	48h	Bloqueja la millora de la DA	
cis-Flupenthixol <u>BL</u> (antagon. DA)	Immed	48h	Bloqueja la millora del clenbuterol	
	Immed	48h	Bloqueja la millora de l'oxotremorina	LaLumiere i McGaugh, 2005
DA <u>BL</u> (agonista DA)	Immed	48h	↑ BL dreta / = BL esquerra	
RU 28362 <u>BL</u> (agonista glucocorticoides)	Immed	48h	↑	Roozendaal i McGaugh, 1997a
	Immed	48h	↑ dosi-dependent	Roozendaal i McGaugh, 1997b
	Immed	48h	↑	Roozendaal i col., 2002
[9-41] α-helical CRH <u>BL</u> (antagon. Hormona alliberadora de corticotropina)	Immed	48h	↓ dosi-dependent	
	3h		=	
	Immed	48h	Bloqueja la millora deguda al clenbuterol	Roozendaal i col., 2008a
			Bloqueja la millora	

				deguda al CRF_{6-33} No afecta la millora del cirazoline No afecta la millora del 8-Br-AMPc	
	CRF_{6-33} BL (inhibidor del lligand del factor alliberador de corticotropina [CRF])	Immed	48h	↑ dosi-dependent	
	Flumazenil BL (antagon. Benzodiacepines)	Immed	48h	↓	Da Cunha i col., 1999
	Muscimol BL (agonista GABAa)	Immed	24h	↓ dosi-dependent	Wilensky i col., 2000
	Lidocaina 2% BL (anestèsic local)	Immed	48h	↓ infusió bilateral = infusió unilat.	LaLumiere i McGaugh, 2005
	8-Br-AMPc BL (anàlog de l'AMPc)	Immed	48h	↑	Roozendaal i col., 2002
		Immed	48h	↑	Roozendaal i col., 2008a
	Gö 7874 BL (inhibidor PKC)	Immed 0.5h		↓ ↓	
	Gö 6976 BL (inhibidor famílies α i BI/BI-PKC)	1.5h 3h 4.5h 6h	24h	= = = =	Bonini i col., 2005
	Relaxin BL (hormona reproductiva)	Immed 3h	48h	↓ dosi-dependent =	Roozendaal i col., 2005
	OFQ/N BL (pèptid opiaci)	Immed 3h 6h	48h	↓ dosi-dependent ↓ dosi-dependent =	Roozendaal i col., 2007
	[Nphe[1]]nociceptin(1-13)NH_2 BL (antagon. OFQ/N)	Immed 3h	48h	↑ dosi-dependent =	
	Noradrenalina BL	Immed	24h	↑ consolidació Reversió dèficits per sevoflurane	Li i col., 2011
EVITACIÓ ACTIVA EN DOS SENTITS	Escopolamina BL (antagonista M1)	Immed	24h	↓ consolidació	Carballo-Marquez i col., 2011
TASQUES DE POR CONDICIONADA					
		24h	14d	↓	Gale i col., 2004
	Lesió NMDA BL (agonista NMDA)	1 d 14d 28d	RT	↓ to i context ↓ to i context ↓ to i context	Maren i col., 1996a
		24h	RT	= efecte del sobreapren.	Maren i col., 1998
		24h	RT	↓	Maren i col., 1999
		Pre-retenció		↓	Muller i col., 1997
CONDICIONAMENT CLÀSSIC DE POR		Pre-retenció		↓	Helmstetter i Bellgowan, 1994
	Muscimol BL (agonista GABAa)	Immed	24h	=	Wilensky i col., 1999
		Immed	24h	=	Wilensky i col., 2000
		Immed	24h	↓	Takahashi i col., 2007
	Lidocaina 2% BL (anestèsic local)	Immed	24h	↓	Huff i col., 2005
		Immed	24h	↓	Vazdarjanova i McGaugh, 1999
	Tetrodotoxina BL (bloqueig canals de	Immed	48h 72h	↓ to i context ↓ to i context	Sacchetti i col., 1999

	Na$^+$)				
	APV BL (antagon. NMDA)	Immed	RT	↓	Maren i col., 1996b
		Pre-retenció		=	
	NBQX BL (antagon. AMPA/Kainat)	Pre-retenció		↓ condic. a la olor = to i context	Walker i col., 2005
	Oxotremorina BL (agonista muscarínic)	Immed	24h	↑	Vazdarjanova i McGaugh, 1999
		Immed	72h	↑	Cangioli i col., 2002
	Atropina BL (antagon. ACh)	Immed	48h	↓ Bloqueja la millora de la dexametasona sistèmica	Power i col., 2000
		Immed	48h	Bloqueja la millora del RU 28362	
	Escopolamina BL (antagon. Muscarínic)	Immed	72h	↓	Passani i col., 2001
	RU 28362 BL (agonista glucocorticoides)	Immed	48h	↑ dosi-dependent	Power i col., 2000
	NA BL (agonista NA)	Immed	24h	↑	Huff i col., 2005
		Immed 3h	48h	↑ =	LaLumiere i col., 2003
	RAMH BL (agonista H$_3$) + Microdiàlisi per Ach	Immed	72h	↑ ↑ alliberació d'ACh al nucli BL	Cangioli i col., 2002
	Immepip BL (agonista H$_3$) + Microdiàlisi per Ach	Immed	72h	↑ ↑ alliberació d'ACh al nucli BL	
	Ciproxifan BL (antagonista H$_3$) + Microdiàlisi per Ach	Immed	72h	↓ ↓ alliberació d'ACh al nucli BL	
	Clobenpropit BL (antagonista H$_3$) + Microdiàlisi per Ach	Immed	72h	↓ ↓ alliberació d'ACh al nucli BL	Passani i col., 2001
	Thioperamide BL (antagonista H$_3$) + Microdiàlisi per Ach	Immed	72h	↓ ↓ alliberació d'ACh al nucli BL	
	Anisomicina BL (inhib. Síntesi de proteïnes)	Immed 14d 6h	24h	↓ reconsolidació ↓ reconsolidació =	Nader i col., 2000
		Immed 6h	24h	↓ dosi-dependent =	Schafe i LeDoux, 2000
	Rp-cAMPS (inhib. PKA)	Immed 6h	24h	↓ dosi-dependent =	
EXTINCIÓ DE LA POR CONDICIONADA	Muscimol BL (agonista GABAa)	Immed	24h	=	Berlau i McGaugh, 2006
		Pre-retenció		↓	Ponnusamy i col., 2007
	NA BL (agonista NA)	Immed	24h	↑ BL dreta	
	Bicuculline BL (antagon. GABA)	Immed	24h	↑ Infusió bilateral i BL dreta	
	Propanolol BL (antagon. β-adrenèrgics)	Immed	24h	=	Berlau i McGaugh, 2006
	Bicuculline + Propanolol	Immed	24h	El propanolol bloqueja la millora de la bicuculline	
	D-cycloserina BL (co-agonista glicina)	1h	24h	↑ Extinció	Ledgerwood i col., 2003

TASQUES OLFACTÒRIES I GUSTATIVES					
TRANSMISSIÓ SOCIAL DE PREFERÈNCIA ALIMENTÀRIA	Muscimol BL (agonista GABAa)	24h	24h	=	Wang i col., 2006
			7d	=	
	Expressió de c-Fos		Imm.	=	Smith i col., 2007
			1 dia	=	
			2 dies	=	
AVERSIÓ CONDICIONADA A UNA OLOR	Tetrodotoxina BL (bloqueig canals de Na$^+$)	Immed	24h	↓	Kilpatrick i Cahill, 2003
	APV BL (antagon. NMDA)	Pre-retenció		=	Walker i col., 2005
	NBQX BL (antagon. AMPA/Kainat)	Pre-retenció		↓	
	Propanolol BL (antagon. β-adrenèrgics)	Immed Pre-RT	72h	= =	Miranda i col., 2007
DISCRIMINACIÓ SIMPLE D'OLORS DISCRIMINACIÓ OLFACTÒRIA	Expressió de c-Fos		1.5 h	↑	Tronel i Sara, 2002
			24h	=	
	Expressió de c-Fos			↑	Hess i col., 1997
POTENCIACIÓ DE L'AVERSIÓ OLFACTÒRIA PER L'ASSOCIACIÓ AMB EL GUST	Muscimol BL (agonista GABAa)	Immed Pre-RT	24h	↓ =	Ferry i col., 1995
	d-APV BL (antagon. NMDA)	Immed Pre-RT	24h	= =	Ferry i Di Scala, 2000
	Expressió de c-Fos	72h	↑ expressió de c-Fos i d'Egr1 al nucli BL		Dardou i col., 2006
	Expressió d'Egr1	72h			
ALTRES TASQUES ASSOCIATIVES					
RECONEIXEMENT D'OBJECTES	NA BL (agonista NA)	Immed	24h	↑ dosi-dependent	Roozendaal i col., 2008b
	Propanolol BL (antagon. β-adrenèrgics)	Immed	24h	↓ dosi-dependent	
		Immed	24h	Bloqueja la millora de la corticoesterona	
		Immed	24h	↑	Roozendaal i col., 2006
	Corticoesterona i.p. + Expressió de pCREB			↑ expressió de pCREB al nucli BL	
PREFERÈNCIA CONDICIONADA L LLOC	Bupivacaina BL (anestèsic local)	Immed 1h Pre-RT	24h	↓ = ↓	Hsu i col., 2002
	Lesió NMDA BL (agonista NMDA)	24 h	7-27d	↓ extinció	Fuchs i col., 2002
	Escopolamina BL (antagon. Muscarínic)	Immed 2h	Imm.	↓ =	Schroeder i Packard, 2002
	Oxotremorina BL (agonista muscarínic)	Immed 2h	Imm.	↑ extinció = extinció	Schroeder i Packard, 2004
	Glucosa BL	Immed 2h	Imm.	↑ extinció = extinció	Schroeder i Packard, 2003
	D-cycloserina BL (co-agonista glicina)	Immed	24h	↑ extinció	Botreau i col., 2006
CONDICIONAMENT INSTRUMENTAL REFORÇAT AMB DROGA	Lesió NMDA BL (agonista NMDA)	24h	7-27d	↓ extinció	Fuchs i col., 2002
TASQUES DE MEMÒRIA DE TREBALL					
LABERINT AQUÀTIC	NA BL (agonista NA)	Immed	24h	↑ dosi-dependent	Hatfield i McGaugh, 1999
	Propanolol BL (antagon. β-adrenèrgics)	Immed	24h	↓	
	APV BL (antagon. NMDA)	Immed	24h	=	Liang i col., 1994

Annex 6. Efectes de diferents tractaments experimentals postentrenament en l'amígdala basolateral sobre la consolidació de diverses tasques.

Annex 7. Estudis experimentals del paradigma DSO

MANIPULACIÓ		RESULTATS	AUTORS
Ventricles laterals			
Timolol (antagon. Recep. β-adrenèrgics) post-adquisició	5 min	= retenció 48h	Sara i col., 1999
	1h	= retenció 48h	
	2h	↓ retenció 48h	
	5h	= retenció 48h	
APV (antagon. Recep. NMDA) post-adquisició	5 min	↓ retenció 48h	Tronel i Sara, 2003
	2h	= retenció 48h	
Còrtex Prelímbic			
Timolol (antagon. Recep. β-adrenèrgics) post-adquisició	5 min	= retenció 48h	Tronel i col., 2004
	2h	↓ retenció 48h	
Microdiàlisi per NA		↑ Alliberació de NA al còrtex PL immediatament i 2 hores després de l'adquisició de la DSO	Tronel i Sara, 2002
Expressió de c-Fos		↑ Expressió de c-Fos 90' després de l'adquisició de la DSO	
Escopolamina pre-adquisició		↓ Retenció i reaprenentatge ↑ nombre d'errors	Carballo-Marquez i col., 2008
Escopolamina immediatament post-adquisició		↓ Consolidació ↑ nombre d'errors i latències	
Escopolamina 1h post-adquisició		= Retenció i reaprenentatge	
Tàlem			
Lesió excitotòxica per NMDA pre-adquisició		∇ adquisició = retenció 24h (assaig 1) ↓ execució en els següents assaigs de la retenció	Quiroz-Padilla i col., 2007
Hipocamp			
APV (antagon. Recep. NMDA) post-adquisició		Infusió post 5': = retenció 48h	Tronel i Sara, 2003
Expressió de c-fos			
CÒRTEX ORBITOFRONTAL		↑ Expressió de c-Fos 90' després de l'adquisició de la DSO	Tronel i Sara, 2002
AMÍGDALA BASOLATERAL		↑ Expressió de c-Fos 90' després de l'adquisició de la DSO	
HABÈNULA LATERAL		↑ Expressió de c-Fos després de la retenció de la DSO	
CÒRTEX INFRALÍMBIC CÒRTEX CINGULAT AMÍGDALA CENTRAL CÒRTEX PIRIFORME		= Expressió de c-Fos després de l'adquisició i la retenció de la DSO	

Annex 7. Efectes de diferents manipulacions experimentals en diferents regions de l'encèfal sobre l'adquisició i retenció de la DSO. [∇: tendència no significativa]

Annex 8. Estudis experimentals de l'hipocamp i la TSPA

ESTRUCTURA	MANIPULACIÓ	TEST	RESULTATS	ESTUDI
Estudis de manipulacions pre-entrenament				
HPCd	Lesió electrolítica	Immed	=	Winocur, 1990
		1 dia	=	
		2 dies	↓	
		4 dies	↓	
		8 dies	↓	
HPC + GD + SUB	Lesió àcid ibotènic	Immed	=	Bunsey i Eichenbaum, 1995
		1 dia	↓	
HPC + GD		Immed	=	
		1 dia	=	
SUB		Immed	=	
		1 dia	=	
HPC + SUB		Immed	=	Burton i col., 2000
		1 dia	=	
HPC		15 min	=	
		7 dies	=	
HPC + SUB	Lesió per aspiració	15 min	↓	Alvarez i col., 2001
		7 dies	↓	
Regió parahipocampal		15 min	↓	
		7 dies	↓	
HPC + SUB	Lesió àcid ibotènic	2.5 h	↓	Alvarez i col., 2002
HPC + GD + SUB		2 dies	↓	Clark i col., 2002
HPC + GD	Lesió NMDA	Immed	=	Winocur i col., 2001
		2 dies	=	
		4 dies	↓	
		8 dies	↓	
CA1	*Knock-out* NMDAR1	1 dia	↓	Rampon i Tsien, 2000
GD	*Knock-out* Thy-1	Immed	↓	Mayeux-Portas i col., 2000
CA1	Deleció Kvβ1.1	Immed	=	Giese i col., 1998
		1 dia	↓	
CA3 i CA1	*Knock-out* per la subunitat α(1B) dels canals de CA^{2+}	1 dia	↓	Jeon i col., 2007
HPC + GD	Infusió HSV-mCREB (sobrexpressió mutant de CREB)	Immed	=	Brightwell i col., 2005
		11 dies	↓	
	Infusió de c-fos antisentit	Immed	=	Countryman i col., 2005a
		14 dies	↓	
CA1d	APV	1 dia	↓	
		3 dies	↓	
Intraperitoneal	CPP MK-801	15 min	=	Roberts i Shapiro, 2002
		1 dia	=	
		2 dies	∇	
		3 dies	↓	
Tot l'encèfal excepte el cerebel	*Knock-out* mGluR1	30 min	=	Kishimoto i col., 2002
		1 dia	↓	
HPC, cerebel i escorces olfactòries	*Knock-out* P311	1 dia	↓	Taylor i col., 2008
Estudis de manipulacions post-entrenament				
HPCd	Lesió electrolítica	Immed — 10 dies	↓	Winocur, 1990
		2 dies — 12 dies	↓	
		5 dies — 15 dies	=	
		10 dies — 20 dies	=	
HPC + GD	Lesió NMDA	1 dia — 11 dies	↓	Winocur i col., 2001
		2 dies — 12 dies	↓	
		5 dies — 15 dies	=	

		10 dies	20 dies	=	
HPC + GD + SUB	Lesió per aspiració	1 dia	11 dies	↓	Clark i col., 2002
		10 dies	20 dies	∇	
		30 dies	40 dies	=	
	Lesió per radiofreqüència	1 dia	30 dies	↓	Ross i Eichenbaum, 2006
		21 dies	30 dies	=	
Intraperitoneal	CPP	Immed	3 dies	=	Roberts i Shapiro, 2002
	MK-801	1 dia		=	

Anàlisis post-entrenament de pCREB			
HPCv	Immed	=	
	1 dia	=	
	2 dies	↑	
GDv	Immed	↑	
	1 dia	=	
	2 dies	↑	Countryman i col., 2005b
HPCd	Immed	=	
	1 dia	=	
	2 dies	=	
GDd	Immed	=	
	1 dia	=	
	2 dies	=	
GDd		=	
CA3d		=	
CA1d		=	
GDv		=	
CA3v		=	
CA1v		=	
GDd	Immed	=	Countryman i Gold, 2007
CA3d		↑	
CA1d		↑	
GDv		↑	
CA3v		↑	
CA1v		↑	

Anàlisis post-entrenament de cFOS			
CA3v	Immed	↑	
	1 dia	=	
	2 dies	↑	
	7 dies	=	
GDv	Immed	↑	
	1 dia	=	
	2 dies	=	
	7 dies	=	
CA1d	Immed	=	
	1 dia	=	
	2 dies	=	Smith i col., 2007
	7 dies	=	
CA3d	Immed	=	
	1 dia	=	
	2 dies	=	
	7 dies	=	
GDd	Immed	=	
	1 dia	=	
	2 dies	=	
	7 dies	=	
SUBv	Immed	↑	Ross i Eichenbaum, 2006
	1 dia	↑	
	2 dies	=	

Estructura	Test	Resultats	Estudi
	21 dies	=	
HPCv	Immed	↑	
	1 dia	=	
	2 dies	↑	
GDv	Immed	↑	
	1 dia	=	
	2 dies	↑	Countryman i col., 2005b
HPCd	Immed		
	1 dia	=	
	2 dies		
GDd	Immed		
	1 dia	=	
	2 dies		

Marcatge de bromode oxiuridina

Estructura	Test	Resultats	Estudi
Marcatge de bromodeoxiuridina	1 sessió d'entrenament: 1 dia	=	
	3 dies	=	
	8 dies	↑	
	2 sessions d'entrenament: 3 dies (després de la 2a sessió)		Olariu i col., 2005
	2 sessions d'entrenament + 2 test: 3 dies (després de la 2a sessió)	↓	

Annex 8. Efectes de diferents tractaments experimentals pre i post entrenament en diferents àrees de l'hipocamp sobre la consolidació de la TSPA. [Immed.: Immediat; ↓: disminueix; =: no afecta;∇: tendència no significativa]

Annex 9. Estudis experimentals d'altres regions cerebrals i la TSPA

ESTRUCTURA	MANIPULACIÓ	TEST	RESULTATS	ESTUDI
Efectes de diferents tractaments experimentals en diferents àrees del PB				
NBM/SI		Immed	=	Berger-Sweeney i col., 2000
		1 dia	↓	
SM/BDBv		Immed	=	
		1 dia	↓	
NBM/SI	Lesió 192 IgG-saporina pre	Immed	↓	
		1 dia	↓	
		3 setmanes	↓	
SM/BDBv		Immed	=	
		1 dia	=	
		3 setmanes	=	Vale-Martínez i col., 2002a
NBM/SI	Lesió 192 IgG-saporina pre (experiència prèvia amb la TSPA)	Immed	=	
		1 dia	=	
SM/BDBv		Immed	=	
		1 dia	=	
NBM/SI	Lesió 192 IgG-saporina pre (experiència prèvia amb la TSPA i 3 opcions de resposta en el test)	Immed	=	
		1 dia	∇	
SM/BDBv		Immed	=	
		1 dia	=	
PB	Infusió i.c.v. 192 IgG-saporina pre (7 dies post-natal)	Immed	=	
		4 hores	↓	
		1 dia (6 mesos post-natal)	↓	Ricceri i col., 2004

NBM	Estimulació elèctrica pre	Immed 1 dia	↑ ↑	Boix-Trelis i col., 2006
NBM/SI	Lesió 192 IgG-saporina post	1 dia 5 dies	11 dies 15 dies	Vale-Martínez i col., 2002a
SM/BDBv		1 dia 5 dies	11 dies 15 dies	

Efectes de diferents tractaments experimentals en l'amígdala				
amígdala	Lesió àcid ibotènic pre	Immed 1 dia	= =	Burton i col., 2000
BL	Infusió muscimol pre	1 dia 7 dies	↓ ↓	Wang i col., 2006
BL	Infusió muscimol 1 dia post	1 dia 7 dies	= =	
BL La Ce	Expressió de c-Fos pre	Immed 1 dia 2 dies	= = =	Smith i col., 2007

Efectes de diferents tractaments experimentals a l'escorça Frontal				
ESCORÇA FRONTAL	Lesió pre	Immed 2 dies 8 dies	= = =	Winocur iMoscovitch, 1999
	Lesió pre (3 opcions de resposta)	Immed 1 dia 2 dies 4 dies 8 dies	= = = ↓ ↓	
ESCORÇA ORBITOFRONTAL	Infusió 192 IgG-saporina pre	2 dies	↓	Ross i col., 2005
CÒRTEX PRELÍMBIC	Infusió d'escopolamina pre	Immed 1 dia	↓ ↓	Boix-Trelis i col., 2007
ESCORÇA FRONTAL	Lesió post	2 dies → 12 dies 5 dies → 15 dies 10 dies → 20 dies	= =	Winocur i Moscovitch, 1999
	Lesió post (3 opcions de resposta	1 dia → 11 dies 2 dies → 12 dies 5 dies → 15 dies 10 dies → 20 dies	↓ ↓ ↓ =	
CÒRTEX ORBITOFRONTAL LATERAL		Immed 1 dia 2 dies 7 dies		
CÒRTEX ORBITOFRONTAL VENTRAL	Expressió de c-Fos	Immed 1 dia 2 dies 7 dies		Smith i col., 2007
CÒRTEX PRELÍMBIC		Immed 1 dia 2 dies 7 dies		
CÒRTEX INFRALÍMBIC		Immed 1 dia 2 dies 7 dies		
ESCORÇA ORBITOFRONTAL	Lesió àcid quinolínic		=	Smith et al. 2010

Efectes de diferents tractaments experimentals al diencèfal				
N. MEDIODORSAL DEL TÀLEM	Lesió pre	Immed 1 dia 2 dies 4 dies 8 dies	= = = = =	Winocur, 1990
N. PARAFASCICULAR	Lesió pre	Immed 1 dia	↓ ↓	Quiroz-Padilla i col., 2006

N. MAMIL·LAR MEDIAL + FEIX	Mutació *Foxb1*		1 dia	=	Radyushkin i col., 2005
N. MEDIODORSAL DEL TÀLEM	Lesió electrolítica post	Immed	10 dies	↓	Winocur i Moscovitch, 1999
		2 dies	12 dies	=	
		5 dies	15 dies	=	
		10 dies	20 dies	=	
Efectes de diferents manipulacions experimentals					
Ratolins GAL-tg			1 dia	↓	Steiner i col., 2001 Wrenn i col., 2002 Wrenn i col., 2003
Knock-out GAL-R1			1 dia	=	Wrenn i col., 2004
Injecció subcutània de APV$_{4-9}$			8 dies	↓	Strupp i col., 1990
			10 dies	=	
			14 dies	↑	
Rates en període proestre			1 dia	↑	Sanchez-Andrade i col., 2005
WAY-200070			Immed	↑	Clipperton i col., 2008
PPT			Immed	=	
			Immed	=	
Mutació CREB			1 dia	↓	Kogan i col., 1997
			1 dia	=	Gass i col., 1998
Restricció de sal en rates Dahl S			5 min	↓	Ruiz-Opazo i col., 2004
			3 hores	↓	
			1 dia	↓	
Knock-out Syt IV			Immed	=	Ferguson i col., 2000
			1 dia	↓	
Knock-out Cplx1			1 dia	↓	Drew i col., 2007
Knock-out Pitx3			1 dia	=	Ardayfio i col., 2008
Sobre expressió NMDA2B				↑	Whit & Youngentob 2004

Annex 8. Efectes de diferents manipulacions experimentals sobre l'adquisició i retenció de la TSPA. [Immed.: Immediat; ↓: disminueix; ↑: augmenta; =: no afecta]