Universitat Autònoma de Barcelona

Modelant la simptomatologia de l'esquizofrènia en les soques de rates romanes i en les genèticament heterogènies NIH-HS

Ignasi Oliveras Puigdellivol

Tesi doctoral:

Modelant la simptomatologia de l'esquizofrènia en les soques de rates romanes i en les genèticament heterogènies NIH-HS

Tesi Doctoral presentada per

Ignasi Oliveras Puigdellivol

per optar al grau de Doctor en Neurociències dins del programa de

Doctorat en Neurociències de la Universitat Autònoma de Barcelona

Aquesta Tesi ha estat supervisada i dirigida per

Alberto Fernández-Teruel

Institut de Neurociències, Departament de Psiquiatria i Medicina Legal, Unitat de Psicologia Mèdica, Facultat de Medicina,

Universitat Autònoma de Barcelona

Maig de 2017

Agraïments

Aquesta Tesi no hauria estat possible sense la capacitat que ha tingut l'Alberto Fernández-Teruel de trobar les idees i conceptes rellevants que s'amagaven darrera dels resultats i de vegades s'amagaven també al fons dels paràgrafs que escrivia durant aquest últim any. Ell ha fet que aquesta Tesi, que a l'inici era un laberint, sigui una descripció coherent i intel·ligible de la meva vida com a "científic" d'aquests darrers tres anys al Departament de Psiquiatria i Medicina Legal. Gràcies per la confiança, l'esforç i la paciència.

També voldria donar les gràcies a la Susana Aznar per haver-me ajudat a adquirir nous coneixements durant els 3 mesos de la meva estada a Copenhague. La feina feta allà no s'hauria pogut realitzar sense l'ajuda del Jonas, el Tomasz, la Luna, la Mille, el Hans Jørgen i el Peter. *Tak*.

Voldria agrair a l'Adolf Tobeña les seves idees i comentaris, sempre interessants, que m'han ajudat molt tant per escriure la Tesi com els articles. També mereixen un gran reconeixement el Javier González-Maeso, l'Osvaldo Giorgi, la Maria G. Corda i la Maria Antonietta Piludu per les seves aportacions i revisions dels articles que formen part de la Tesi.

Vull donar les gràcies als companys del laboratori: Ana, Cristóbal, Carles, Dani, Toni, Gloria, Esther, Cristina, Aitor, perquè amb la vostra ajuda, al llarg d'aquests anys, he quedat sorprès de la quantitat i sobretot de la qualitat de la feina que em fet plegats durant aquests anys. Els encerts i virtuts d'aquesta Tesi es deuen a vosaltres i els errors i mancances (probablement) són cosa meva.

També voldria agrair a la Beatriz Molinuevo i al Joan Taberner l'oportunitat de tenir les primeres experiències com a "professor". I també agrair a totes les persones que han treballat o treballen al departament (Igor, Jaume, Magda, Rafael, Rosa Maria, Lydia,...) de totes elles he aprés i han creat un ambient molt agradable i amable on m'he sentit molt a gust aquests anys.

Per acabar voldria donar les gràcies als meus amics de Manresa (Licel, Marina, Abraham, Anna, Mireia, Sergi, Jordi, Míriam, Laura,...) pel seu interès i per ajudar-me a "desconnectar" durant els caps de setmana. També voldria reconèixer la gran feina que ha fet el Salvador amb la portada que resumeix molt bé la Tesi.

Agrair a la meva família el suport perquè durant aquest anys hi ha hagut moments de tots colors i vosaltres heu estat al meu costat amb un somriure i amb ganes de tirar endavant.

Tots vosaltres (i altres persones, que per culpa de la meva mala memòria no us he mencionat, i us demano disculpes) formeu part d'aquesta Tesi i espero que us pugui interessar, una mica.

Aquesta Tesi ha estat finançada amb els projectes PSI2013-41872-P (MINECO), 2014SGR-1587 (DGR) i la beca predoctoral FI (DGR 2014)

“The truth will set you free. But not until it is finished with you.”

David Foster Wallace, Infinite Jest

Índex

Abreviatures

2AR-mGluR2: Complex format pels receptors 5-HT2A de serotonina i mGlu2 de glutamat

5-CSRTT: 5 choice serial reaction time task

5-HT: Serotonina

5-HT1A: Receptor de serotonina del tipus 1A

5-HT2A: Receptor de serotonina del tipus 2A

5-HT2C: Receptor de serotonina del tipus 2C

ACTH: Hormona adrenocorticotropa

APO-SUS/APO-UNSUS: soques de rates seleccionades per la seva sensibilitat (i la manca de sensibilitat) a l'apomorfina

AS: Aïllament social

ATV: Àrea tegmental ventral

COMT: Catecol-O-metil Transferasa

CPF: Còrtex prefrontal

CPFDL: Còrtex prefrontal dorsolateral

D1: Receptor de dopamina tipus 1

D2: Receptor de dopamina tipus 2

DA: Dopamina

DISC1: Disrupted in Schizophrenia 1

DMPT: Delayed matching to place task

DMTS: Delayed matching to sample

DOI: 2,5-Dimetoxi-4-iodoamfetamina

DSM: Diagnostic and Statistical Manual of Mental Disorders

GABA: àcid γ-aminobutíric

HTR1A: Gen del receptor de 5-Hidroxitriptamina (Serotonina) 1A

HTR2A: Gen del recepto de 5-Hidroxitriptamina (Serotonina) 2A

KO: Knockout

LPS: Lipopolisacàrid

LSD: Dietilamida d'àcid lisèrgic

MATRICS: Measurement and Treatment Research to Improve Cognition in Schizophrenia

mGluR2: Receptor metabotròpic de glutamat tipus 2

MK-801: Dizocilpina

MWM: Morris water maze

nAcc: Nucli Accumbens

NH: Neonatal handling

NIH-HS: National Institutes of Health N/Nih Genetically Heterogeneous Rat stock

NMDAR: Receptor N-metil-D-aspartat

NR1: Subunitat 1 del receptor ionotròpic de glutamat NMDA

PCP: Fenciclidina

Poly I:C: Àcid poliriboinosinic:poliribocitidilic

PPI: Prepulse inhibition

RHA/RLA: Roman high avoidance i Roman low avoidance

RT-qPCR: Reverse transcription quantitative polymerase chain reaction

SNC: Sistema nerviós central

RESUM

En aquesta Tesi es presenten quatre estudis que tenien l'objectiu de validar les rates romanes (RHA-I i RLA-I) com a model animal d'alguns símptomes rellevants per a l'esquizofrènia. L'Estudi 1 tenia com a objectiu principal fer la caracterització conductual de les rates romanes d'alta i baixa evitació (RHA-I i RLA-I, respectivament) i de les rates genèticament heterogènies NIH-HS en dues tasques relacionades amb els símptomes de l'esquizofrènia, com són els dèficits de filtratge atencional, mesurats amb la inhibició de la resposta de sobresalt per un prepols (PPI), i els dèficits de memòria de treball. A l'Estudi 2 es van investigar els efectes de l'aïllament social, sobre diverses conductes/fenotips: el filtratge atencional (PPI), l'ansietat, la hiperactivitat motora i la memòria. A l'Estudi 3 es van avaluar els efectes de substàncies antipsicòtiques (haloperidol i clozapina) i propsicòtiques (apomorfina, MK-801, DOI) sobre la PPI i l'activitat motora. Finalment, el quart estudi tenia l'objectiu d'estudiar les diferències d'expressió gènica de HTR1A i HTR2A entre les soques romanes, i entre rates NIH-HS seleccionades pel seus nivells extrems de PPI.

Els resultats de l'Estudi 1 mostren que les rates RHA-I, comparades amb les RLA-I i les NIH-HS, tenen dèficits de PPI i de memòria de treball, i que les rates NIH-HS presenten valors alts (semblants als de les rates RLA-I) tant de PPI com de memòria de treball. A més a més, els resultats mostren correlacions positives entre la PPI i la memòria de treball. A l'Estudi 2, les rates RHA-I aïllades socialment manifesten dèficits de PPI, augment de l'ansietat, hiperactivitat i dèficits d'aprenentatge. A l'Estudi 3 vam observar que les rates RHA-I són més sensibles als efectes dels agonistes/antagonistes dopaminèrgics (apomorfina/haloperidol) sobre la PPI. Per altra banda, les rates RLA-I són més sensibles als efectes dels fàrmacs agonistes/antagonistes serotoninèrgics (DOI/clozapina) i a l' antagonista NMDA (MK-801) sobre la PPI. Els resultats de l'Estudi 4 mostren diferències entre soques en l'expressió gènica al còrtex prefrontal (dels gens HTR1A i HTR2A) i a l'estriat (del gen HTR2A), on les rates RHA-I mostren un increment de l'expressió en ambdós casos. Les rates NIH-HS seleccionades pels seus nivells de PPI també mostren diferències d'expressió del gen HTR1A al còrtex prefrontal. Globalment, els estudis augmenten la validesa aparent (Estudi 1 i 2), la validesa predictiva (Estudi 3) i la validesa de constructe (Estudi 4 i el 2 en menor mesura) de les rates RHA-I com a model animal d'alguns dels símptomes relacionats amb l'esquizofrènia.

ABSTRACT

In this Thesis we present four studies that were aimed at validating the Roman rat strains (RHA-I and RLA-I) as an animal model of some schizophrenia-relevant symptoms. Study 1 was devoted to behaviorally characterize the Roman high- (RHA-I) and low-avoidance (RLA-I) rats, and the genetically heterogeneous rats (NIH-HS), in two tasks related to some schizophrenia-relevant symptoms, i.e. prepulse inhibition of the startle response (PPI, a measure of sensorimotor gating) and spatial working memory measured in the Morris water maze. In Study 2, we investigated the effects of chronic social isolation on several behaviours/phenotypes in the Roman rats: sensorimotor gating (PPI), anxiety, locomotor hyperactivity and spatial memory. In Study 3 we evaluated the effects of several antipsychotics (haloperidol and clozapine) and propsychotics (apomorphine, DOI and MK-801) on PPI and locomotor activity. Finally, Study 4 was aimed to evaluate whether there are differences in gene expression (of the HTR1A and HTR2A genes) between the Roman rat strains, and to see if these differences also appear between NIH-HS rats selected for their extremely high or low levels of PPI.

The results of Study 1 show that RHA-I rats have PPI and working memory deficits and that NIH-HS rats present high levels of PPI and working memory. There are positive associations between PPI and working memory, since rats with high PPI (RLA-I and NIH-HS) also have a better performance in the working memory task. In Study 2, the socially-isolated RHA-I rats exhibit PPI deficits, increased anxiety, locomotor hyperactivity and spatial learning deficits. Study 3 shows that RHA-I rats are more sensitive to the effects of dopamine agonists/antagonists (apomorphine/haloperidol) on PPI, whereas RLA-I rats are more sensitive to the effects of serotonin agonists/antagonists (DOI/clozapine) and to the NMDA antagonist (MK-801) in the PPI test. The results of Study 4 show differences in gene expression between the Roman rats in the prefrontal cortex (HTR1A and HTR2A genes) and in the striatum (HTR2A gene), with RHA-I rats having enhanced expression of these genes. The NIH-HS rats selected for their extreme PPI levels also show differences of expression of the HTR1A gene in the prefrontal cortex. As a whole, the studies of the present Thesis increase the apparent validity (Study 1 and 2), the predictive validity (Study 3) and construct validity (Study 4 and Study 2 to a lesser extent) of the RHA-I rats as an animal model of some of the symptoms related to schizophrenia.

I. Introducció

1. Aspectes generals de l'esquizofrènia

L'esquizofrènia és un trastorn mental que afecta al voltant d'un 1% de la població (Ross et al., 2006; Tamminga i Holcomb, 2005). El primer autor que va descriure els símptomes, diagnòstic i les possibles causes de l'esquizofrènia va ser Emil Kraepelin al 1893. Ell va anomenar aquest trastorn *dementia praecox* i el va definir com un trastorn crònic amb un inici florit, que s'inicia al final de l'adolescència, que produeix discapacitat funcional i social, i que sol tenir un mal pronòstic. Va distingir tres tipus: hebefrènic, catatònic i paranoide (Ion i Beer, 2002). Per la seva banda, Eugen Bleuler va ser el primer a usar el terme esquizofrènia (1911), i va descriure el trastorn com una patologia dissociativa, posant l'accent en els símptomes negatius (Bleuler, 1950). Per la seva banda, Schneider va posar èmfasis en els símptomes positius (Schneider, 1959).

L'inici de l'esquizofrènia tendeix a aparèixer entre els 18-25 anys en homes i 25-30 anys en dones, i la simptomatologia, sobretot la positiva, es presenta en forma de brots que ocorren al llarg de la vida del pacient. Els símptomes es poden diferenciar en tres tipus:

1) *Positius*.- Impliquen un augment o excés de les funcions cerebrals normals. En aquesta categoria s'inclouen els deliris, al·lucinacions, trastorns del pensament, desorganització de la conducta i del llenguatge. Els pacients en els que predomina la simptomatologia positiva responen bé a la medicació, i quan aquests símptomes remeten poden portar una vida normal (Tandon et al., 2013)
2) *Negatius*.- Es refereixen a una disminució o dèficit d'algunes funcions, sobretot relacionades amb les emocions i la motivació. En aquest grup s'inclouen l'anhedònia, l'abúlia, l'apatia, l'aïllament social, etc. Els pacients en els que predomina aquest tipus de simptomatologia tendeixen a patir un deteriorament prolongat, i la medicació actual tendeix a no tenir efectes positius en aquests pacients.
3) *Cognitius*.- En aquest grup s'inclouen totes les funcions cognitives en les que s'ha observat un dèficit en els pacients esquizofrènics de forma consistent. Aquestes funcions cognitives són: l'atenció/vigilància, memòria de treball, memòria/aprenentatge verbal, memòria/aprenentatge visual,

funcions executives i atencionals, la cognició social, la rapidesa de processament i el raonament i solució de problemes.

Els símptomes cognitius, tot i que no estan presents al criteri A del DSM-V (*Diagnostic and Statistical Manual of Mental Disorders*; American Psychiatric Association, 2013), si que són una característica molt important del trastorn, malgrat que, com discuteix Tandon et al. (2013), no distingeixen a l'esquizofrènia d'altres trastorns en els que els dèficits cognitius també hi apareixen. També s'ha de tenir en compte que durant el curs de la malaltia els símptomes positius i negatius van fluctuant, mentre que els dèficits cognitius tenen una estabilitat al llarg del temps, i solen ser aparents en pacients abans del primer brot psicòtic (Harvey et al., 2003). Les dades mostren que els pacients amb esquizofrènia tenen uns dèficits cognitius/intel·lectuals equivalents a una desviació estàndard (o més) de la mitjana de la població general (van Os i Kapur, 2009). Els dèficits cognitius normalment són independents dels símptomes psicòtics i sovint no mostren cap millora, fins i tot quan els símptomes psicòtics ja han remès completament (van Os i Kapur, 2009).

En l'àmbit clínic, per poder diagnosticar l'esquizofrènia es segueixen els criteris diagnòstics recollits en les diferents versions del *Diagnostic and Statistical Manual of Mental Disorders* (DSM; American Psychiatric Association, 2013). Per poder diagnosticar el trastorn s'han de complir 3 dels símptomes més rellevants (positius i negatius) durant més d'un mes. Els altres criteris es refereixen als efectes, a la vida social i laboral del pacient, a la durada de les alteracions provocades pels símptomes (6 mesos com a mínim) i al diagnòstic diferencial amb altres trastorns amb els quals l'esquizofrènia comparteix alguns dels seus símptomes (trastorn esquizoafectiu, trastorns de l'estat d'ànim o trastorns relacionats amb l'addicció a les drogues, etc; veure revisió de Tandon et al. 2013, per veure els canvis introduïts a l'última versió del DSM-V). Recentment s'ha publicat la cinquena versió del DSM, i comparant-la amb la quarta podem veure que s'han introduït canvis, relacionats amb l'augment de la utilitat clínica, la validesa o la millor concordança amb la definició d'esquizofrènia de la *International Classification of Diseases* (Gaebel et al., 2013; ICD, World Health Organization, 1992).

En l'àmbit de la recerca bàsica és molt important tenir models animals que reprodueixin els fenotips i (en la mesura del coneixement científic disponible) mecanismes neurobiològics que són característics del trastorn, per tal de millorar els tractaments

d'aquests símptomes. No obstant, s'ha de tenir en compte l'alt grau de comorbiditat que hi ha entre trastorns. Per exemple, els pacients esquizofrènics presenten una alta comorbiditat amb trastorns relacionats amb l'ansietat i la depressió, un 15% presenten atacs de pànic, un 29% té un trastorn d'estrès posttraumàtic, un 23% té un trastorn obsessiu compulsiu, mentre que un 50 % presentarà un diagnòstic de depressió i un 47 % patirà un trastorn relacionat amb l'abús de substancies (Buckley et al., 2009, Figura 1). També s'ha de tenir en compte que els trastorns mentals i els criteris diagnòstics han anat variant al llarg dels anys (Gøtzsche 2014, 2015). Aquestes característiques fan que les avaluacions psiquiàtriques tinguin un caràcter subjectiu i que l'eficàcia dels tractaments sigui molt dubtosa (Gøtzsche 2014, 2015). Per tant, es fa molt difícil desenvolupar models animals fiables per tal d'incrementar el coneixement de la neurobiologia dels trastorns mentals com l'esquizofrènia.

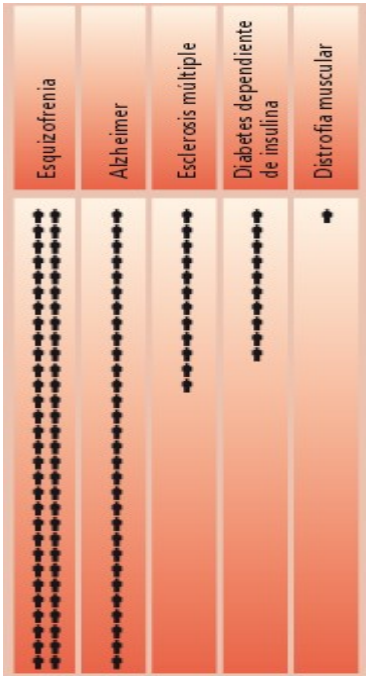


Figura 1.- *Prevalença de cassos d'Esquizofrènia (0.5-1.5%), Alzheimer, Esclerosis Múltiple, Diabetis i Distrofia Muscular en la població general. Extret de Moreno et al. (Mente y cerebro, 44: 18-27, 2010a).*

1.1 Teoria dopaminèrgica de l'esquizofrènia

La primer teoria formulada per tal d'explicar l'etiologia de l'esquizofrènia va ser la teoria dopaminèrgica. Hi ha quatre vies dopaminèrgiques. La primera va des de l'àrea tegmental ventral (ATV) fins al nucli accumbens (nAcc) i està relacionada amb els símptomes positius del trastorn. Per altra banda, hi ha una altra població de neurones amb els seus cossos neuronals també a l' ATV i que projecta cap al còrtex frontal, que està relacionada amb la simptomatologia negativa i cognitiva del trastorn. Molts autors han proposat que en l'esquizofrènia hi ha una disminució de dopamina (DA) al còrtex frontal, mentre que hi ha un augment de DA al sistema límbic. Així, s'ha formulat la hipòtesi que els símptomes negatius estarien provocats per aquesta hipofrontalitat mentre que la hiperactivitat dopaminèrgia del sistema límbic explicaria els símptomes positius (Howes et al., 2012).

Les dues altres vies, una que projecta les neurones des de la *substantia nigra* cap a l'estriat i l'altra que va des del hipotàlem fins a la glàndula pituïtària, estan implicades en els efectes secundaris dels fàrmacs antipsicòtics. Concretament la via nigro-estriada està relacionada amb efectes adversos de tipus motor, provocats pels fàrmacs que tenen la seva diana terapèutica en els receptors de dopamina D2 i que produeixen els anomenats efectes extrapiramidals. Per la seva banda, la via tuberoinfundibular és la responsable dels efectes secundaris com la hiperprolactinèmia (Howes et al., 2012)

Actualment es coneixen dues famílies diferents de receptors de DA. La família dels receptors D1, que inclou el receptor D1 i el D5, i la família del receptor D2, que inclou els receptors D2, D3 i D4. Respecte a la seva distribució al sistema nerviós central (SNC), els receptors de la família D1 es trobarien localitzats al còrtex prefrontal (CPF) i, per la seva banda, els receptors de la família D2 es troben en regions subcorticals. Pel que fa als efectes terapèutics dels antipsicòtics típics (p. ex. haloperidol, clorpromacina, pimocida, tioridacina, etc.), s'ha vist que l'antagonisme dels receptors D2 al sistema límbic provoca una reducció dels símptomes positius, mentre que l'acció d'aquesta fàrmacs en altres regions subcorticals (glàndula pituïtària, ganglis basals, etc.) provoca l'aparició dels efectes secundaris (Moreno et al., 2010a).

S'ha observat que en pacients esquizofrènics l'administració d'amfetamines (que potencien la funció dopaminèrgica) provoca un augment dels símptomes positius causat per un augment anormal de DA, així com per l'alteració del metabolisme de DA

presinàptica d'aquests pacients (Abi-Dargham et al., 2009; Howes et al., 2012; Kegeles et al., 2002, 2010; Laruelle et al., 1996, 2003). També tenen la densitat de receptors D2 elevada a l'estriat. De tota manera, sembla que aquests canvis dopaminèrgics en els pacients podrien ser deguts al fet que estan reben tractament amb antipsicòtics, ja que no s'han confirmat en pacients que no havien rebut cap tipus de medicació (Steeds et al., 2015).

1.2 Teoria serotoninèrgica de l'esquizofrènia

Aquesta teoria es basa en les observacions de subjectes sota als efectes d'agonistes 5-HT2A/C (psilocibina o la dietilamida d'àcid lisèrgic –LSD-), que provoquen al·lucinacions i alteracions de la percepció i cognició semblants als que apareixen a les fases inicials dels trastorns psicòtics com l'esquizofrènia (Vollenweider i Geyer, 2001). Pel que fa a les al·lucinacions provocades pels agonistes 5-HT2A en humans, en general són de tipus visual, mentre que les al·lucinacions més usuals en pacients psicòtics són les de tipus auditiu. Tot i això, els efectes neuroquímics que provoquen aquestes substàncies són molt similars als que apareixen en els pacients esquizofrènics (Steeds et al., 2015).

Els agonistes 5-HT2A/C tenen validesa com a inductors de símptomes relacionats amb l'esquizofrènia, ja que provoquen dèficits que són similars als que trobem en pacients esquizofrènics. Per exemple, l'administració d'aquestes substàncies a persones sense el trastorn o als pacients amb esquizofrènia, provoca dèficits a l'hora de filtrar estímuls irrellevants. En aquest sentit, dos testos que mostren aquests dèficits són l'habituació davant d'estímuls de sobresalt i la inhibició de la resposta de sobresalt per un prepols (PPI). La percepció del temps també està alterada cosa que provoca problemes a l'hora de planificar accions. La relació entre la serotonina (5-HT) i l'esquizofrènia s'ha vist recolzada per diferents observacions, com per exemple el fet que la reserpina (*Rauwolfia serpentina*) té propietats antipsicòtiques. També per l'aparició dels antipsicòtics atípics, com ara la clozapina, olanzapina o la risperidona, que actuen com antagonistes del receptor 5-HT2A i del receptor de dopamina D2, i que a part de presentar efectes antipsicòtics no provoquen els efectes extrapiramidals que sí mostren els antipsicòtics típics (Moreno et al., 2010a).

Per tant, la teoria serotoninèrgica de l'esquizofrènia està recolzada pels estudis amb agonistes 5-HT2A que simulen la fase primerenca i aguda del trastorn, caracteritzada per les al·lucinacions (Geyer i Vollenweider, 2008, Halberstadt i Geyer, 2013), tot i que en dosis altes els al·lucinògens provoquen també alguns dels símptomes negatius (aïllament social i catatonia). A més a més, els estudis amb els antipsicòtics atípics, que són antagonistes del receptor 5-HT2A (entre d'altres), també mostren la implicació del sistema de neurotransmissió serotoninèrgica en la patofisiologia de l'esquizofrènia (Baou, et al., 2016; Moreno et al., 2010a)

1.3 Teoria glutamatèrgica de l'esquizofrènia

La teoria glutamatèrgica ha servit per explicar els dèficits cognitius que també són característics de l'esquizofrènia. La teoria glutamatèrgica de l'esquizofrènia es basa en els efectes que tenen els antagonistes del receptor N-metil-D-aspartat (NMDAR) com la fenciclidina (PCP), la ketamina o la dizocilpina (MK-801), sobretot pel que fa als símptomes negatius i dèficits cognitius.

El fet que l'administració aguda d'antagonistes NMDAR com la PCP o la ketamina, en humans sense el trastorn, produeixi símptomes com hiperactivitat, deliris, al·lucinacions, trastorns del pensament i dèficits cognitius (Neill et al., 2010), va deixar clar que la desregulació de la neurotransmissió mitjançada pels receptors NMDA intervé en l'aparició dels símptomes. Al contrari que en la teoria dopaminèrgica, la varietat de símptomes (positius, negatius i cognitius) induïts pels antagonistes NMDAR és molt més àmplia. Segons Kantrowitz i Javitt (2010) els receptors NMDA són el punt de convergència en el que múltiples factors, tant ambientals com genètics, podrien estar actuant per predisposar a un individu a l'esquizofrènia.

El paper dels receptors NMDA en els símptomes positius, segons Kantrowitz i Javitt (2010), es relacionaria amb el fet que la hiperactivitat dopaminèrgica límbica podria ser conseqüència d'un dèficit del funcionament de neurones GABAèrgiques corticals (vegeu Figura 2). Això provocaria una menor inhibició (per part d' aquelles interneurones GABAèrgiques) de les neurones glutamatèrgiques piramidals que, per tant, estimularien en excés les neurones dopaminèrgiques de la via mesolímbica (vegeu Figura 2).

La desregulació del sistema glutamatèrgic també podria explicar les diferències que es troben en el volum d'algunes regions cerebrals. No obstant, segons Steeds et al. (2015) la hipofunció dels receptors NMDA és una observació que en pacients esquizofrènics no és gaire consistent, però si que hi ha treballs que mostren aquesta característica, així com la reducció de la subunitat NR1 del receptor (Steeds et al., 2015, Figura 2).

Un altre aspecte que dóna suport a la implicació del glutamat en la patofisiologia de l'esquizofrènia és el fet que l'excés de DA a les àrees mesencefàliques i mesolímbiques per si sol no pot explicar tot el ventall de símptomes cognitius que exhibeixen els pacients amb el trastorn. Segons Neill et al. (2010), l'organització i funcions del sistema glutamatèrgic al prosencèfal, a més a més de la relació entre gens que han estat associats amb l'esquizofrènia, com la neuroregulina, disbindina, la COMT i el DISC1, podrien estar relacionats amb la desregulació del sistema glutamatèrgic en aquest trastorn (Harrison i Owen, 2003; Harrison i Weinberger, 2005) i amb la presència dels símptomes cognitius de l'esquizofrènia (Kristiansen et al., 2007).

No obstant, s'ha de tenir en compte que una teoria o hipòtesis per si sola mai podrà explicar tota la simptomatologia dels trastorns psicòtics i en especial de l'esquizofrènia. A més a més de les teories de la fisiopatologia de l'esquizofrènia comentades a dalt n'hi ha d'altres en que es destaquen altres sistemes de neurotransmissió, com el GABAèrgic, colinèrgic, endocanabinoid, etc. Per tant, des d'un punt de vista teòric i pràctic la integració de les diverses teories pot ajudar a descriure millor la patofisiologia de l'esquizofrènia (Jentsch i Roth, 1999, Figura 2).

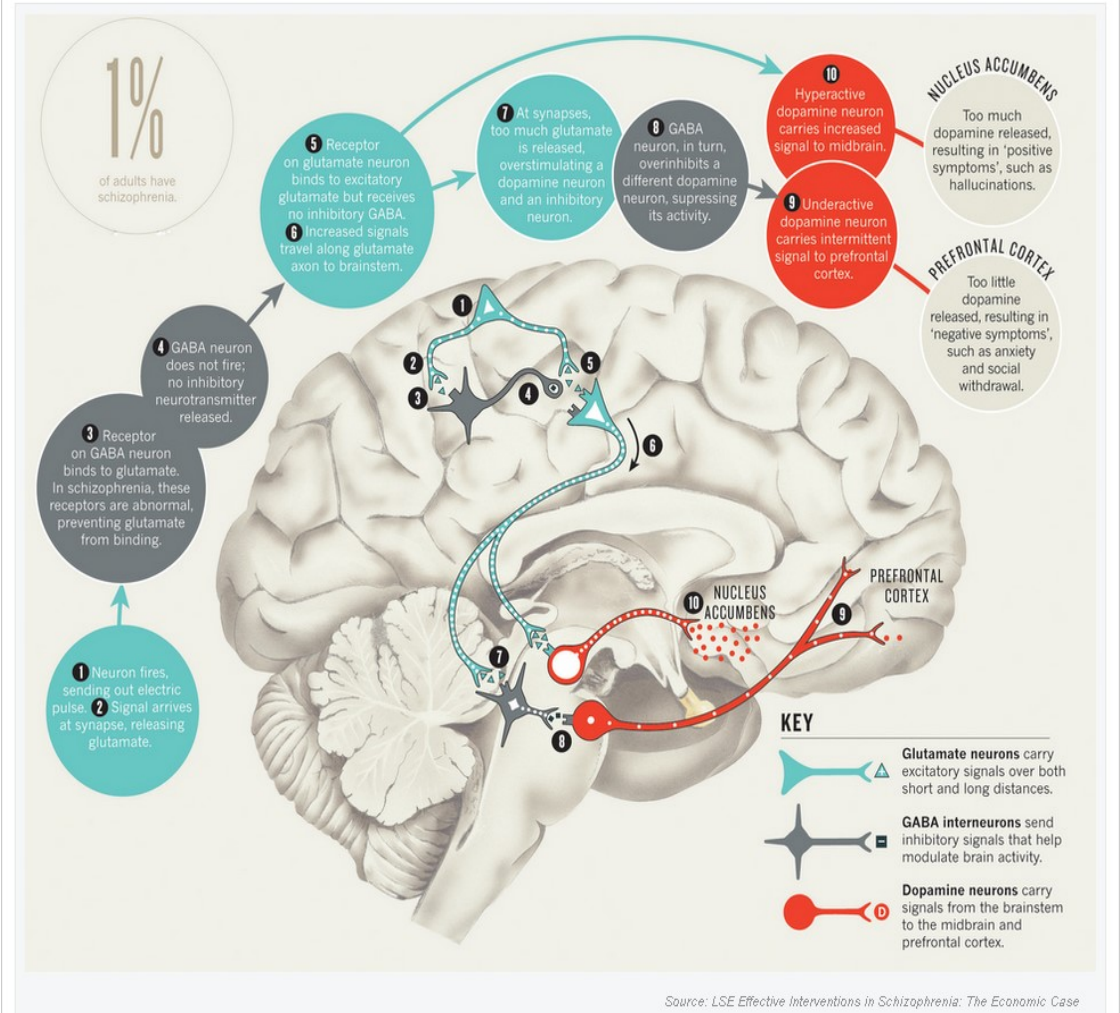


Figura 2.- *Vies implicades en la neurofisiologia de l'esquizofrènia: 1) potencial d'acció; 2) el senyal arriba a la sinapsi glutamatèrgica; 3) els receptors postsinàptics que estan a les neurones GABAèrgiques no funcionen correctament en l'esquizofrènia; 4) per tant, es produeix una desinhibició ja que les neurones GABAèrgiques no transmeten la seva inhibició; 5 i 6) les neurones glutamatèrgiques que no han rebut el fre de les neurones GABAèrgiques transmeten al seu senyal fins al tronc de l'encèfal; 7 i 8) l'excés de glutamat provoca un augment de l'activitat de les neurones dopaminèrgiques de la via mesolímbica i un augment de la funció de les neurones GABAèrgiques que inhibeix la via mesocortical de dopamina; 9) la reducció de dopamina al còrtex prefrontal provoca l'aparició de símptomes negatius com l'ansietat o l'aïllament social; 10) L'augment de dopamina al nAcc provoca els símptomes positius com les al·lucinacions. Extret d'Elert (Nature 508, S2–S3, 2014).*

1.4 Neuroanatomia de l'esquizofrènia

El còrtex prefrontal (CPF) està relacionat amb moltes funcions (percepció, atenció, llenguatge, intel·ligència, memòria, consciència, etc.), i també amb les conductes de tipus social (Fuster, 2009; Goldberg, 2009). El CPF ha evolucionat amb la finalitat de relacionar la informació sensorial i motora per tal de dur a terme respostes flexibles davant de l'entorn canviant que ens envolta. El CPF rep projeccions de diverses àrees subcorticals per tal d'integrar informació rellevant per portar a terme accions planificades i deliberades que tenen un objectiu. Per tal de fer tot això, el CPF és la part més important del cervell pel que fa a les funcions executives (memòria de treball, inhibició de respostes, presa de decisions, atenció, etc.).

La part dorsolateral del CPF (CPFDL) s'encarrega de moltes funcions cognitives, i ha estat objecte de molts estudis que han vist que tant l'estructura com la connectivitat d'aquesta regió amb l'hipocamp estan alterats en persones amb esquizofrènia. Per exemple, s'ha observat, que en tasques de memòria de treball, l'activació del CPFDL està disminuïda en pacients esquizofrènics (Meyer-Lindenberg, 2010).

La funció del CPF s'ha relacionat amb els símptomes negatius de l'esquizofrènia, ja que les lesions al CPF provoquen dèficits semblants a aquests símptomes (dèficits d'atenció, falta d'*insight*, etc.). A més a més, aquests símptomes correlacionen amb el volum del CPF en pacients esquizofrènics segons alguns estudis (Wible et al., 1995, 2001). El CPF també participa en la regulació d'altres àrees subcorticals que estan implicades en els símptomes positius de l'esquizofrènia (Frith et al., 2009; Goldberg 2009). De fet, en pacients esquizofrènics s'ha trobat una activació menor del CPF, tot i que no de manera consistent (Hill et al., 2004).

Pel que fa a les diferències estructurals més robustes que s'han trobat comparant els pacients esquizofrènics respecte a la població general, s'ha vist que els primers presenten un engrandiment dels ventricles laterals i del tercer ventricle, del *cavum septum pellucidi* i un volum cerebral menor. Altres autors també han descrit un engrandiment dels solcs dels lòbuls temporal i frontal, així com una reducció de la matèria grisa dels mateixos lòbuls. Altres estudis han mostrat que l'hipocamp i les estructures del solc temporal superior estan reduïdes en pacients esquizofrènics, i que aquesta reducció està associada amb la presència d'al·lucinacions (Tamminga i Holcomb, 2005). També s'han trobat

anormalitats estructurals en els ganglis bassals, tàlem, cerebel i cos callós (Antonova et al., 2004; Honea et al., 2005; Niznikiewicz et al., 2003).

Els estudis de neuroimatge han mostrat que les estructures del lòbul temporal com l'amígdala, hipocamp, còrtex entorrinal i el gir temporal superior, presenten disminucions de volum en pacients esquizofrènics, sobretot a l'hemisferi esquerre (McCarley et al., 1999; Shenton et al., 1992, 1997). Aquestes estructures temporals estan interconnectades amb el CPF, i s'ha vist una alta correlació entre el volum del CPF i les estructures esmentades (Goldman-Rakic et al., 1984; Pandya i Yeterian, 1990; Wible et al., 1995). Segons Wible et al. (2001) aquestes correlacions són signe d'un sistema funcional patològic del cervell "esquizofrènic".

1.5 Neuroanatomia funcional de l'esquizofrènia

Diferents estudis han avaluat la relació entre els símptomes de l'esquizofrènia i l'activitat cerebral mitjançant estudis de neuroimatge funcional (Figura 3). Per exemple, s'ha vist que el còrtex prefrontal dorsolateral (CPFDL) està relacionat amb les funcions executives, i la seva activitat està alterada en l'esquizofrènia, on hi predominen conductes desorganitzades a causa de la inhabilitat del CPFDL per suprimir comportaments inapropiats en conductes dirigides a un objectiu, així com també a les conductes autodirigides (referides a l'habilitat de regular i adaptar el comportament a una situació per tal d'assolir els nostres objectius; Goghari et al., 2010). Per altra banda, la part ventrolateral del CPF ha estat associada als símptomes negatius durant tasques que avaluen les funcions executives. L'activitat anormal del CPF medial i l'amígdala estan relacionats amb un augment de la paranoia durant el processament d'estímuls emocionals (Goghari et al., 2010). L'activitat anormal del CPF medial està relacionada amb els dèficits en els judicis de caràcter social mentre que l'activitat de l'amígdala estaria relacionada amb els dèficits de reconeixement de les emocions i les interpretacions errònies de situacions ambigües. L'hipocamp i el gir parahipocampal també estan relacionats amb el processament d'estímuls emocionals i amb els símptomes positius com la paranoia. Concretament l'hipocamp participa en la regulació d'estats emocionals involucrats en generar conductes en situacions amenaçadores o potencialment amenaçadores. L'estriat ventral està associat amb els símptomes negatius durant tasques

de reforç i condicionament, el que estaria relacionat amb que la motivació està regulada per aquesta area (Goghari et al., 2010).

Totes aquestes diferències podrien tenir el seu origen en el neurodesenvolupament pre- i perinatal, que podria provocar un augment de la vulnerabilitat del cervell durant el neurodesenvolupament postpubertal i que, conjuntament amb altres factors ambientals (estrès, drogues, etc) que estan associats amb l'inici del trastorn, provocarien seqüeles que a llarg termini portarien a la neurodegeneració (Kristiansen et al., 2007). No obstant, els resultats són controvertits pel que fa a la reducció del metabolisme de les regions frontals, ja que hi ha estudis que no han trobat diferències i d'altres que han trobat un augment (Kristiansen et al., 2007). També s'ha de tenir en compte que l'ús d' antipsicòtics provoca una disminució del metabolisme de les àrees frontals (Kristiansen et al., 2007).

Pel que fa a les diferències de l'hipocamp, s'ha vist que en pacients esquizofrènics el pol anterior està reduït i és metabòlicament més actiu que en la població general (Heckers i Konradi, 2010; Small et al., 2011). A més a més, s'ha observat que un augment del metabolisme hipocampal està relacionat amb un augment de DA, que segons la literatura provocaria l'aparició dels símptomes positius (Lodge i Grace, 2008; Modinos et al., 2015; Winton-Brown et al., 2014). Els estudis d'imatge per Ressonància Magnètica funcional (RMf) també han mostrat patrons d'activació anormals de l'hipocamp durant tasques de memòria en pacients esquizofrènics (Heckers i Konradi, 2010).

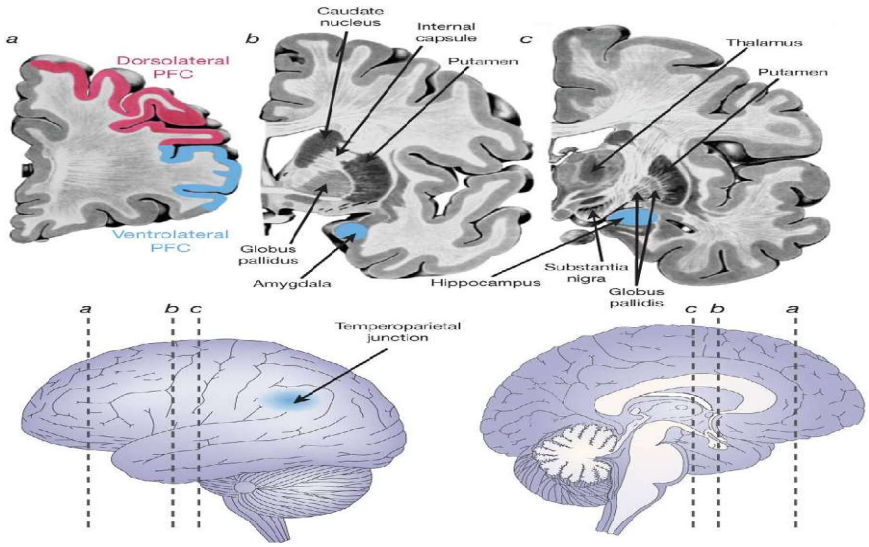


Figura 3.- *Àrees que estan involucrades a la neuropatofisiologia de l'esquizofrènia. Extret de Meyer-Lindenberg (Nature 468, 194-202, 2010).*

2. Tractaments farmacològics de l'esquizofrènia: antipsicòtics típics i antipsicòtics atípics

El tractament del trastorn és una part molt important per tal que el pacient pugui desenvolupar-se en l'àmbit social i laboral amb normalitat. De tota manera, s'ha de tenir en compte que al voltant d'un terç de pacients que rep antipsicòtics aconsegueix revertir els símptomes, un altre terç té una millora parcial, i el terç restant no té cap tipus de millora amb els antipsicòtics actuals (Meltzer, 1997; Mosolov et al., 2012). Els antipsicòtics típics provoquen els efectes secundaris extrapiramidals i el parkinsonisme, mentre que els atípics provoquen augment de pes i trastorns metabòlics (Abbott, 2010; Lieberman et al., 2005).

El desenvolupament dels antipsicòtics des del 1950 ha tingut moments en que la sort ha tingut un paper rellevant. Per exemple, és el cas del descobriment de la clorpromazina com antipsicòtic, ja que un histaminèrgic derivat de la fenotiazina, la prometazina, induïa símptomes de quietud eufòrica i un estat d'indiferència en pacients no psiquiàtrics, mentre que en pacients que havien estat sotmesos a cirurgia induïa calma amb somnolència i *relax* (Geyer et al., 2012, Taula1). Un molècula semblant, la clorpromazina, va ser desenvolupada i testada per Laborit et al., (1952), i a partir d'aquest fet el tractament de l'esquizofrènia va canviar de manera dràstica (Geyer et al., 2012,

Geyer i Ellenbroek, 2003). A partir de les següents dècades es van anar desenvolupant els anomenats antipsicòtics típics o neurolèptics, com l'haloperidol. Aquests antipsicòtics van fer que els pacients fossin més manejables i tranquils. Malgrat aquests avantatges els antipsicòtics típics no són eficaços per tractar els símptomes negatius com l'anhedònia o la manca de motivació. A més a més, aquest tipus d'antipscòtics tenen molts efectes indesitjables, com ara el parkinsonisme o la distonia.

Dins dels antipsicòtics de segona generació, o antipsicòtics atípics, la clozapina és l'exemple paradigmàtic (Figura 4 i Taula 1). Aquesta classe d'antipscotics (que inclou la risperidona, olanzapina, quetiapina, ziprasidona, aripiprazola, etc) tenen l'avantatge de no provocar els efectes secundaris dels antipsicòtics típics. Tot i això, els seus efectes de possible millora dels símptomes negatius i dels dèficits cognitius són encara molt controvertits, i segons Geyer et al. (2012) no afegeixen cap millora en aquest sentit. A més a més, aquests antipsicòtics també provoquen efectes secundaris, com increment de pes i trastorns metabòlics, entre d'altres.

Taula 1.- *Evolució dels antipsicòtics utilitzats pel tractament de l'esquizofrènia des dels antipsicòtics típics o de primera generació (en els que s'inclou l'haloperidol), els antipsicòtics atípics o de segona generació com la clozapina, els antipicòtics atípics amb menys efectes secundaris que la clozapina com l'olanzapina i, finalment, a l'any 2007 es van començar a investigar els fàrmacs que tenen com a diana molecular el receptor mGlu2 i que alguns estudis mostren que milloren alguns símptomes negatius. Modificat de Moreno et al. (Mente y cerebro, 44: 18-27, 2010a).*

	Classificació	**Exemples**	**Mecanismes**	**Eficàcia**	**Efectes secundaris**
Anys 50	Típics	Haloperidol Clorpromazina	Antagonisme D2	Redueix símptomes positius però no els negatius	Símptomes extrapiramidals, Parkinsonisme, distonia muscular
Anys 70	Atípics	Clozapina	Antagonisme D2 i 5-HT2A	Redueix símptomes positius i alguns de negatius; és eficaç en esquizofrènies resistents al tractament	Menys símptomes Parkinsonians; símptomes metabòlics que limiten molt el seu ús
1995	Nous atípics	Olanzapina, Risperidona Quetiapina	Similars als de la clozapina	Semblant a la de la clozapina però no són tan eficaços en les esquizofrènies resistents al tractament	Menys efectes secundaris que la clozapina
2007	Glutamatèrgics	LY404039	Activació del receptor metabotròpic mGlu2	Redueix símptomes positius i alguns de negatius	Sense efectes secundaris coneguts

3. Estat actual dels tractaments farmacològics de l'esquizofrènia

Respecte a l'efectivitat dels antipsicòtics típics, l'haloperidol ha estat comparat amb d'altres de la seva mateixa classe ("típics"), com ara el bromperidol, loxapina o la trifluoperazina. En la metanàlisi de Samara et al. (2015) es mostra que els efectes terapèutics de l'haloperidol no eren diferents dels altres antipsicòtics, mentre que respecte dels efectes secundaris l'haloperidol provocava una acatisia menys severa que els altres antipsicòtics a mig termini.

Un altre antipsicotic típic molt usat és la clorpromazina. Una revisió de Saha et al. (2016) ha mostrat que l'olanzapina és més eficaç que la clorpromazina. Tot hi això la metanàlisi té les dades de diferents estudis majoritàriament realitzats en població asiàtica, i que tenen diferents febleses de tipus metodològic que fan que les seves conclusions no siguin definitives.

Pel que fa a les metanàlisis on s'han comparat diferents antipsicòtics atípics, com és la metanàlisi fet per Asenjo Lobos et al. (2010), aquests autors mostren que la clozapina no és millor comparant-la amb altres fàrmacs d'aquesta família. Concretament, la clozapina només es va mostrar estadísticament millor que la zotepina pel que fa a l'estat mental general, però no van trobar diferències significatives amb altres antipsicòtics com l'olanzapina o la risperidona. Per altra banda, dos estudis xinesos van trobar que la quetiapina era més eficaç que la clozapina a l'hora de millorar els símptomes negatius. Respecte als efectes secundaris, la clozapina va provocar menys efectes indesitjables que la risperidona. No obstant, els pacients esquizofrènics tractats amb clozapina van mostrar un decrement més gran de glòbuls blancs que els pacients tractats amb olanzapina, i una hipersalivació i sedació més gran que els pacients tractats amb olanzapina, risperidona i quetiapina (Asenjo Lobos et al. 2010). El tractament amb clozapina també va provocar més convulsions que l'olanzapina i la risperidona. Finalment, la clozapina també va provocar un augment de pes significativament més gran que el provocat per la risperidona (Asenjo Lobos et al., 2010).

Per altra banda, també hi ha metanàlisis que mostren que la clozapina és l'antipsicòtic atípic més efectiu que hi ha (Leucht et al., 2013), encara que també és dels antipsicòtics amb més efectes secundaris de tipus metabòlic (agranulocitosi, anèmia aplàsica). A més a més, s'ha de tenir en compte que en aquesta metanàlisi es van excloure els pacients en els que predominaven els símptomes negatius, els pacients refractaris i els pacients estables. També s'ha de tenir molt en compte que hi ha altres antipsicòtics amb efectes secundaris que poden causar problemes cardíacs (que poden acabar amb la mort prematura). A la metanàlisi de Leucht et al. (2013), per exemple, antipsicòtics com el sertindole, ziprasidona o l'amisulpride van causar problemes cardíacs.

Per tant, veient els resultats obtinguts a molts estudis es fa evident la necessitat de trobar noves dianes moleculars per tal de millorar els efectes que s'obtenen amb els tractaments actuals, ja que la majoria d'ells no provoquen millores dels dèficits cognitius i símptomes

negatius, i tenen efectes indesitjables greus. Un camí que pot aportar alguna millora en aquest sentit són les substàncies que tenen la seva diana molecular en els receptors glutamatèrgics. Per exemple, a la metanàlisi de Tiihonen i Wahlbeck (2006) conclouen que algunes substàncies com la D-serina o la glicina (que interactuen amb els receptors NMDA glutamatèrgics) redueixen els símptomes negatius, encara que l'efecte és petit i els estudis que ho mostren tenen algunes mancances metodològiques (Tiihonen i Wahlbeck, 2006).

A part de les substàncies que interactuen amb el receptors NMDA, diferents treballs de González-Maeso (2007, 2008) han ajudat a augmentar el coneixement que tenim dels receptors metabotròpics de glutamat (en especial el receptor mGlu2), així com respecte de la seva relació amb la simptomatologia de l'esquizofrènia (Ellaithy et al., 2015; Muguruza et al., 2016). A més a més, s'ha vist que les àrees cerebrals que expressen aquests receptors coincideixen amb les àrees que expressen el receptor de serotonina 5-HT2A. Això ha provocat que la recerca de nous antipsicòtics es centrés en aquest receptor de glutamat durant els últims anys (Taula 1). En aquest sentit diferents assaigs clínics han mostrat resultats força positius pel que fa als moduladors positius del receptor mGlu2 (Kinon et al., 2013; Liu et al., 2012; Patil et al., 2007).

Tenint en compte l'ampli ventall d'efectes indesitjats que provoquen els tractaments farmacològics actuals, es fa molt important desenvolupar nous models animals que puguin ajudar a incrementar els coneixements que es tenen sobre la neurobiologia dels diferents tipus d'esquizofrènies, o de símptomes concrets del trastorn, per tal de tenir tractaments millors i més específics. En aquest sentit, el treball d'aquesta Tesi, enfocada als models animals (en rates) que presenten diferències innates en el complex format pel receptor 5-HT2A i el mGlu2, anomenat "2AR-mGluR2" (González-Maeso et al. 2008), pensem que pot aportar informació significativa sobre la relació entre els sistemes serotoninèrgics i glutamatèrgics i alguns símptomes/dèficits rellevants per l'esquizofrènia que també són presents en els models utilitzats (vegeu més endavant).

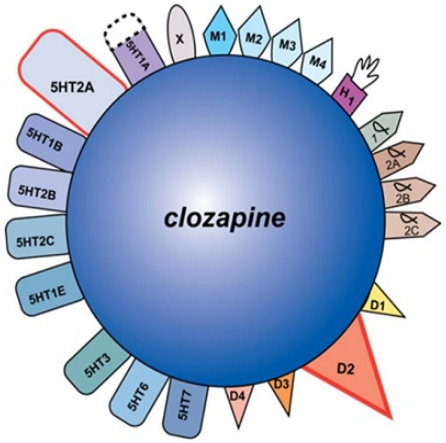


Figura 4.- *Els diferents tipus de receptors serotoninèrgics, dopaminèrgics, adrenèrgics, colinèrgics i histaminèrgics amb els quals la clozapina interactua. Els més importants són els receptors D2 i 5-HT2A, que estan implicats amb els seus efectes terapèutics. Extret de Stahl (Antipsychotic Agents, Capítol 11; 401-458, 2000).*

4. Consideracions sobre els models animals de l'esquizofrènia

El desenvolupament dels models animals de diferents trets de personalitat, de símptomes de trastorns i de psicopatologies, ha servit per augmentar el coneixement que tenim de les bases psicològiques i neurobiològiques del comportament normal i dels trastorns mentals. Per tal de fer aquesta funció, els models animals s'han de fonamentar, en la mesura que es pugui, en la patogènesis que té una patologia en humans (Tobeña i Fernández-Teruel, 2010; Nestler i Hyman, 2010). En el cas de l'esquizofrènia, per tal d'explicar l'etiologia es parla de les interaccions entre els gens i l'ambient, és a dir factors genètics que augmenten el risc de patir la malaltia i estressors ambientals que augmenten també la vulnerabilitat (Jones et al., 2011; Sawa i Snyder, 2002).

Un model animal idealment ha d'aportar almenys tres tipus principals de validesa per tal que se'l consideri òptim per poder avaluar els efectes de nous fàrmacs ens assaigs clínics, millorar el coneixement de les bases neurobiològiques del trastorn o símptomes, etc. (p. ex. Escorihuela i Fernández-Teruel 1998; Van Haaren 1995; Willner 1991). Aquestes tres valideses són:

1) *Validesa aparent.*- Es refereix a que la majoria de símptomes que es donen en humans també han d'aparèixer al model animal, tot i que cal tenir en compte que alguns símptomes són impossibles de modelar en animals, com poden ser les al·lucinacions i deliris en l'esquizofrènia.
2) *Validesa predictiva.*- Es refereix a que els fàrmacs (o d'altres tractaments) que ajuden a revertir els símptomes d'un trastorn en humans també ho haurien de fer en el model animal i, al contrari, els fàrmacs que no tenen efectes (o que empitjoren) en un trastorn tampoc haurien de mostrar efectes (o haurien d' empitjorar) en el model animal.
3) *Validesa de constructe.*- Es refereix a les bases neurobiològiques que es coneixen de la malaltia també han d'estar presents al model animal. Per tant, segons Jones et al. (2011), per tal de complir aquests criteris un bon model animal de l'esquizofrènia, per exemple, hauria de mostrar les següents característiques: mostrar els dèficits després de la pubertat, pèrdua de connectivitat i funcionalitat a l'hipocamp i al còrtex, alteracions dopaminèrgiques i glutamatèrgiques, vulnerabilitat davant situacions estressants, alteracions de la resposta als reforçaments, dèficits cognitius i alteracions de la conducta social.

Els models animals permeten fer procediments invasius de diferents tipus per tal d'introduir canvis moleculars o estructurals que poden estar relacionats amb l'etiologia del trastorn. Segons Jones et al. (2011) els models animals de l'esquizofrènia es poden classificar en: models lesionals, models genètics, models del neurodesenvolupament i models farmacològics.

Els models lesionals van ser desenvolupats per tal de contrastar la hipòtesi que l'esquizofrènia és un trastorn en que hi ha neurodegeneració. No obstant, la majoria de treballs amb aquests models han focalitzat la seva atenció en la hipòtesi que els estressors pre- i perinatals provoquen alteracions en el desenvolupament cerebral, en concret de les fases de mort i de poda cel·lular i també en la connectivitat neuronal (Marcotte et al., 2001). Respecte als models farmacològics, parteixen de la hipòtesi que en l'esquizofrènia hi ha alteracions importants en diversos sistemes de neurotransmissió. Els models farmacològics tendeixen a garantir la validesa predictiva, mentre que el seu grau de validesa de constructe i aparent no és tan alt. Els models genètics també tenen en compte el paper important del neurodesenvolupament, però posen l'èmfasi en els factors de susceptibilitat genètica. Aquests models podrien ajudar a descobrir nous biomarcadors

genètics de l'esquizofrènia, com podria ser el decrement d'immunoreactivitat de la parvalbúmina de les interneurones corticals (Sawa, 2009)

Els dèficits cognitius i els símptomes negatius són els més resistents als fàrmacs actuals, de manera que els models animals haurien de permetre descobrir noves dianes terapèutiques per tal de millorar la cognició dels pacients. Dins d'aquestes noves dianes trobem diferents receptors dopaminèrgics (D4 y D1), els receptors α7-nicotínics, receptors muscarínics, i altres receptors glutamatèrgics (mGlu2), serotoninèrgics (5-HT1A, 5-HT2A) i cannabinoids. No obstant, l'heterogeneïtat de dianes, com també l'heterogeni ventall d'escales i tests que es fan servir tant en humans com en rosegadors per avaluar els símptomes, augmenten la dificultat per trobar fàrmacs adequats per augmentar la cognició d'aquests pacients (Geyer et al., 2012; Steeds et al., 2015).

Per tal de millorar l'efectivitat dels tractaments sobre els dèficits cognitius i els símptomes negatius hi ha hagut diferents iniciatives, enfocades a identificar els símptomes o fenotips més rellevants o nuclears, per tal d'obrir el camí per desenvolupar noves teràpies farmacològiques. Dins d'aquestes iniciatives trobem la *Measurement and treatment research to improve cognition in schizophrenia* (MATRICS), en que es van descriure diferents processos cognitius claus per tal de poder avaluar els dèficits que normalment es presenten en l'esquizofrènia. En humans aquestes característiques cognitives claus són: els dèficits d'atenció/vigilància, de memòria de treball, de memòria i aprenentatge lingüístics, de memòria i aprenentatge visuals, de cognició social, de rapidesa de processament i de raonament i solució de problemes. Per tal de modelar aquestes dimensions i dèficits en animals hi ha diferents tests. Un exemple, segons McLean (2010), són les tasques de *Delayed Matching to sample task* (DMTS), en les que a l'animal se li presenta un estímul "mostra" i al cap d'uns segons (retard) se li presenten dos estímuls (un d'igual a l'estímul de mostra i un de diferent). L'animal ha d'escollir l'estímul que és igual a la mostra i rebrà un reforç. Aquesta tasca correspon a les diferents escales del WAIS que mesuren memòria de treball.

Per altra banda, la inhibició de la resposta de sobresalt per un prepols (PPI) també és un dels tests que forma part dels mètodes per avaluar l'atenció/vigilància. Aquestes dues tasques la PPI i el DMTS, han estat destacades i rellevants per tal de poder modelar fenotips importants en els models animals d'esquizofrènia. En la present Tesi s'han fet servir la PPI i una tasca de DMPT (*Delayed matching to place task*) al laberint aquàtic de

Morris (MWM), dues tasques que estan altament relacionades amb les habilitats cognitives que segons la iniciativa MATRICS s'han d'avaluar i que podran permetre desenvolupar noves teràpies farmacològiques per tal de revertir els dèficits cognitius associats a l'esquizofrènia. Altres tasques com el *reversal learning, attentional set-shifting* o *novel object recognition* també han estat tasques de destacada importància a l'hora de desenvolupar un model animal vàlid. El *reversal learning* explora la capacitat que tenen els animals d'inhibir aprenentatges anteriors, *l'attentional set shifting* avalua la capacitat de formar estratègies atencionals a partir d'unes regles, i el *novel object recognition*, que com a mesura de la memòria de treball fa ús de la tendència natural dels animals a explorar estímuls nous (McLean, 2010). Totes les tasques esmentades a dalt presenten un bon grau de validesa aparent quan s'avaluen en models del neurodesenvolupament, genètics o farmacològics de símptomes esquizofrènics, doncs les funcions representades per aquestes tasques són deficitàries en aquests models, com també ho són en pacients esquizofrènics.

Per tal que hi hagi un nivell alt de translacionalitat entre el treball en neurociència bàsica i el treball clínic de neuròlegs, psiquiatres i psicòlegs, els endofenotips poden ser una bona eina per aconseguir millorar els tractaments (Bender et al., 2007). Els endofenotips són trets que es poden mesurar de forma sensible, que tenen una alta validesa de constructe perquè (suposadament) estan més a prop dels mecanismes neurobiològics rellevants (Bender et al., 2007). En el cas de l'endofenotip anomenat "inhibició de la resposta de sobresalt per un prepols (PPI)", el procediment que es fa servir en humans per mesurar i quantificar la PPI és molt similar al dels rosegadors. Els pacients esquizofrènics presenten dèficits de PPI (semblants als observats després d'administrar un psicoestimulant dopaminèrgic en rates), tot reforçant la seva validesa aparent.

Reforçant el seu valor com a endofenotip, també és important tenir en compte el fet que la PPI correlaciona amb altres característiques (p. ex. Vargas et al. 2016). Per exemple s'ha observat que el CPF és molt important per tenir una PPI normal, i que, per tant, disfuncions del CPF i el conseqüent deteriorament de la PPI i d'altres processos atencionals (com la inhibició latent) poden ser trets característics de trastorns mentals, diferents dels que es basen en tests/questionaris fets pels pacients o familiars (Vargas et al., 2016).

4.1 Models de dèficits en funcions específiques: Dèficits atencionals

Per tal de modelar els dèficits de filtratge atencional s'han fet servir diferents tests conductuals, el més usual és la inhibició de la resposta *startle* per un prepols (PPI). La inhibició de la resposta *startle* per un prepols fa referència a la reducció de la resposta del reflex de sobresalt quan un estímul intens, que provoca sobresalt, està precedit per un altre estímul poc intens i que no provoca la resposta de sobresalt. Els dèficits de PPI, com els que són presents en l'esquizofrènia, estan associats a la "inundació" d'estímuls sensorials (irrellevants) i la conseqüent fragmentació cognitiva (McGhie i Chapman, 1961)

Els primers estudis en els que es van valorar els circuïts implicats en la resposta de sobresalt van ser portats a terme per Michael Davis i els seus col·laboradors durant els anys 70 i 80 (Davis, 1984). Pel que fa als primers estudis que relacionaven els dèficits de PPI amb l'esquizofrènia van ser treballs realitzats al laboratori d' Enoch Callaway (Braff et al., 1978).

Els primers autors que van estudiar els efectes de fàrmacs (usualment antipsicòtics) i drogues sobre la PPI són de Swerdlow i col·laboradors (1986), que van valorar els efectes de l'apomorfina sobre la PPI en rates hipersensibles a les substàncies dopaminèrgiques. Pel que fa als agonistes serotoninèrgics, els primers estudis que van mostrar els dèficits provocats per aquestes substàncies van ser els de Mansbach i col·laboradors (1989a-b) i el de Rigdon i Weatherspoon (1992). Aquests estudis van mostrar que els dèficits de PPI estaven mediats per els receptors de serotonina 5-HT1 i 5-HT2.

Els efectes dels antagonistes no-competitius del receptor N-metil-D-aspartat (NMDAR) han estat estudiats des de fa molts anys ja que provoquen l'aparició de símptomes psicòtics i provoquen un empitjorament dels pacients esquizofrènics. A més a més, diferents estudis mostren que, en rosegadors, els antagonistes NMDAR provoquen dèficits i fenotips semblants als que presenten els pacients amb esquizofrènia, entre ells el deteriorament de la PPI. A més a més, aquests resultats estan en consonància amb la hipòtesi hipoglutamatèrgica de l'esquizofrènia (Geyer et al 2001).

Des de la publicació de Swerdlow et al. (1992) molts estudis han mostrat que els dèficits de PPI estan presents en molts trastorns, i en aquella publicació ja s'hipotetitzava que els dèficits de PPI són una part important de malalties com el síndrome de la Tourette, la

malaltia de Huntington o el trastorn obsessiu-compulsiu. També es deia que la participació del circuït cortico-estriato-palido-talàmic estava involucrat tant en la modulació de la PPI com en la patofisiologia d'aquests trastorns (Swerdlow et al., 1987). Una de les característiques importants de la PPI és el fet que és un procés que se'l considera un endofenotip (*fentotip intermedi*), que facilita poder modelar els aspectes genètics, moleculars i fisiològics implicats en l'endofenotip (Figures 5 i 6). A més a més, els endofenotips com la PPI poden predir altres fenotips més complexos. Per exemple l'execució en la PPI està correlacionada amb les funcions executives (Bitsios et al., 2006). També han mostrat que la PPI correlaciona positivament amb els bons resultats que dóna la teràpia cognitiva-conductual per a la psicosis (Fendt i Koch, 2013). No obstant, hi ha autors que afirmen que la PPI no seria un bon biomarcador, ja que hi ha diferents estudis en que no s'han trobat correlacions positives entre la PPI i altres mesures (Koch, 2013; Fendt i Koch, 2013). Per això, segons Fendt i Koch (2013), la PPI no seria un bon biomarcador que pugui predir el curs clínic de l'esquizofrènia, el pas de la fase prodròmica al primer brot, o que pugui predir quines persones tenen un risc més alt de patir el trastorn. Per Swerdlow et al. (2008) la PPI és un fenotip quantitatiu fiable que pot servir per explorar les bases neurobiològiques i genètiques dels dèficits de filtratge atencional que mostren els pacients esquizofrènics. Per tant, es pot dir que els models animals de PPI tenen un cert grau de validesa aparent (Swerdlow et al., 2008), validesa predictiva (Geyer et al., 2001) i, en menor mesura, validesa de constructe (Kohl et al. 2013).

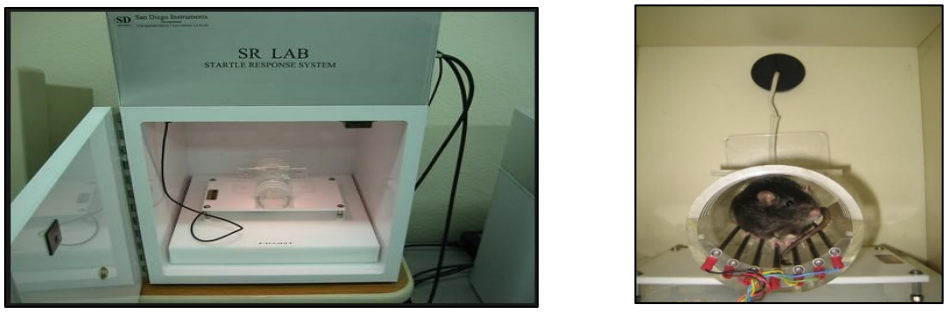


Figura 5 i 6.- *Imatges on es mostren les caixes d'"startle" que s'utilitzen per realitzar la PPI*

Altres tests que es fan servir per mesurar l'atenció són la inhibició latent, que es refereix al procés pel qual un estímul presentat repetidament perdrà força per actuar com a estímul condicionat en un futur aprenentatge (és a dir, aquest serà més difícil d'adquirir pel fet d'usar un estímul condicionat al que el subjecte ha estat repetidament pre-exposat abans d'iniciar-se l'aprenentatge) (Figura 7). S'ha vist que en pacients esquizofrènics aquesta habituació als estímuls no es produeix, i la inhibició latent està deteriorada (Geyer i Moghaddam, 2002). Una altra manera d'avaluar l'atenció en humans és a través del potencial evocat P50, que està alterat en pacients esquizofrènics. En persones sense el trastorn, quan es presenten dos estímuls sonors separats per 500 ms, es produeix una reducció (filtratge) del potencial evocat (P50) després del segon estímul. En animals, una mesura anàloga d'aquest paradigma, és el potencial evocat (N40) que es genera a l'hipocamp, 40 ms després d'haver estat exposat als estímuls sonors (Geyer i Moghaddam, 2002).

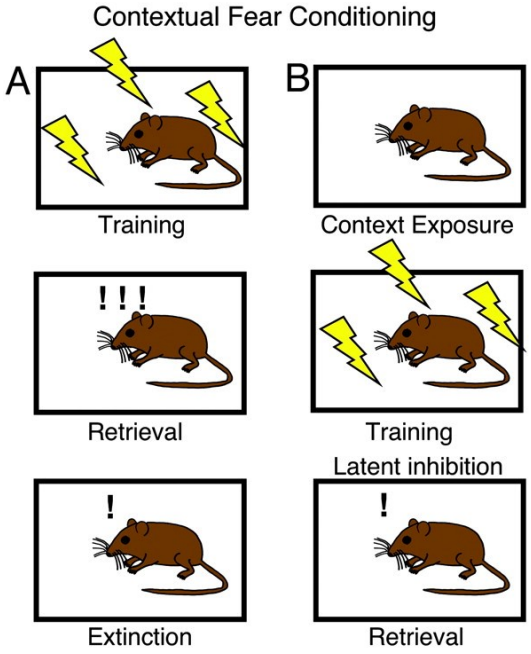
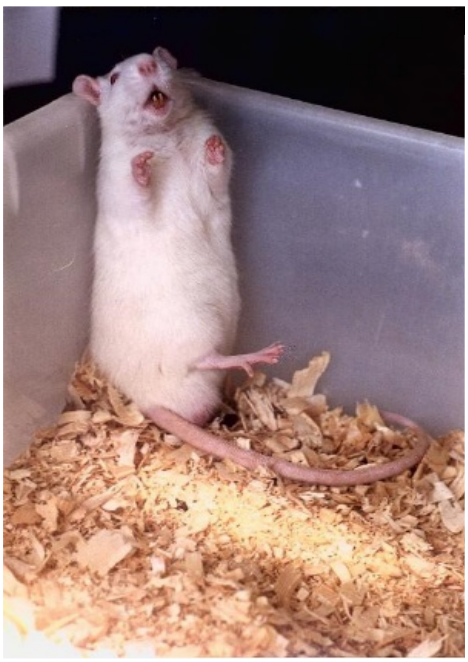


Figura 7.- *Esquema en el que es representa un procediment de la inhibició latent, en el que la pre-exposició al context (B) provoca que en una sessió posterior (training, B), en la que s'emparella el context amb un estímul aversiu (una descàrrega elèctrica, B), l'aprenentatge associatiu (condicionament clàssic de por al context) costi més d'establir, i per això els animals que prèviament han estat pre-exposats al context (B) mostren respostes condicionades de por menors (compareu fase "retrieval" de B i A) que els animals que no han estat pre-exposats (A) al context. Extret de Garelick i Storm (PNAS, 102(26): 9091–9092, 2005).*

4.2 Models de dèficits en funcions específiques: Activitat locomotora

L'avaluació de l'activitat motora ha servit per avaluar els efectes dels psicoestimulants dopaminèrgics, que provoquen hiperactivitat, així com els efectes dels antipsicòtics que indueixen hipoactivitat i antagonitzen els efectes dels psicoestimulants (Geyer i Moghaddam, 2002). La validesa aparent pel que fa als símptomes rellevants per l'esquizofrènia dels tests que mesuren l'activitat no recau tant en la hiperactivitat sinó en les conductes estereotipades i perseveratives, que també es presenten en pacients esquizofrènics. Per altra banda, els tests que mesuren activitat també han servit per avaluar diferents fàrmacs per tractar el trastorn bipolar, ja que en aquest trastorn hi ha un augment de l'activitat locomotora (Geyer, 2008; Figures 8 i 9). En general, els resultats dels experiments que han avaluat els efectes dels agonistes dopaminèrgics i els antagonistes NMDAR han mostrat que, en ambdós casos, hi ha un augment de l'activitat locomotora, fet que aporta validesa aparent i predictiva als models animals. A més a més, els antipsicòtics redueixen la hiperactivitat provocada pels agonistes dopaminèrgics i els antagonistes NMDAR (Sams-Dodd, 1998, van den Buuse, 2010), el que dóna encara més suport a la validesa predictiva d'aquests models.



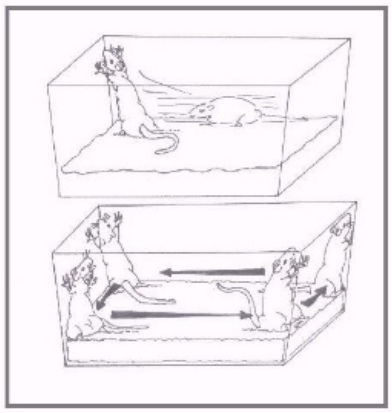


Figura 8 i 9.- *Diferents tipus de conductes motores i estereotípies que estan relacionades amb els símptomes positius en els models animals de l'esquizofrènia.*

4.3 Models de dèficits en funcions específiques: Cognició

Els dèficits cognitius són molt rellevants, ja que són els que ens permeten diferenciar l'esquizofrènia del trastorn bipolar o d'altres trastorns psicòtics, i també tenen un impacte molt gran en el funcionament social i laboral dels pacients esquizofrènics.

Desenvolupar tasques anàlogues en rosegadors, que permetin avaluar aquests dèficits, ha sigut molt difícil, i la majoria d'esforços s'han centrat en tests per mesurar la memòria de treball, el canvi d'estratègies o la flexibilitat conductual. Per avaluar la memòria de treball es fan servir tasques anomenades *delayed matching or non-matching to sample* (Kesner et al., 1996) i el *discrete trial delayed alternation* (Aultman et al., 2001). Per avaluar el canvi d'estratègies s'utilitzen laberints en que les rates han de fer servir, primer, estratègies relacionades amb una resposta motora (p. ex. girar sempre a la dreta) i, després, fer servir estratègies de tipus visual per tal d'obtenir un reforçament. Aquests tipus de tasques permeten estudiar diferents tipus de processos cognitius com la flexibilitat intra i entre-estratègies, o la perseveració. Un altre test important per avaluar aquestes habilitats és el *Five-Choice Serial Reaction Time task* (5-CSRTT; Figura 10), dissenyat per Robbins i col·laboradors (Robbins et al., 1989), que està basat en el test *Continuous Performance Test of Attention* que s'usa en humans (Mirsky et al., 1960), i que consisteix en que l'animal ha d'estar atent a un estímul visual (un llum) que pot aparèixer en cinc zones on hi ha uns forats on l'animal ha d'introduir el morro, per tal de

rebre el reforç a la menjadora. En aquesta tasca es pot avaluar la impulsivitat, la perseveració, les omissions, l'atenció, etc. dels animals (Geyer i Moghaddam, 2002). Globalment, la validesa aparent d'aquests models és alta, perquè són coneguts els dèficits cognitius dels pacients esquizofrènics, i perquè als darrers anys el ventall d'aquests dèficits que s'han pogut modelar amb animals ha augmentat (Geyer i Moghaddam, 2002). Respecte a la validesa predictiva i la de constructe cal dir que la pràctica totalitat dels antipsicòtics (aguts o crònics) s'ha vist que empitjoren o no afecten les funcions cognitives en els models animals esmentats (Young et al., 2009). En aquest sentit, aquesta evidència experimental coincidiria força amb els resultats observats en pacients esquizofrènics, en els que els tractaments antipsicòtics, com a norma, no milloren o fins i tot empitjoren les funcions cognitives.

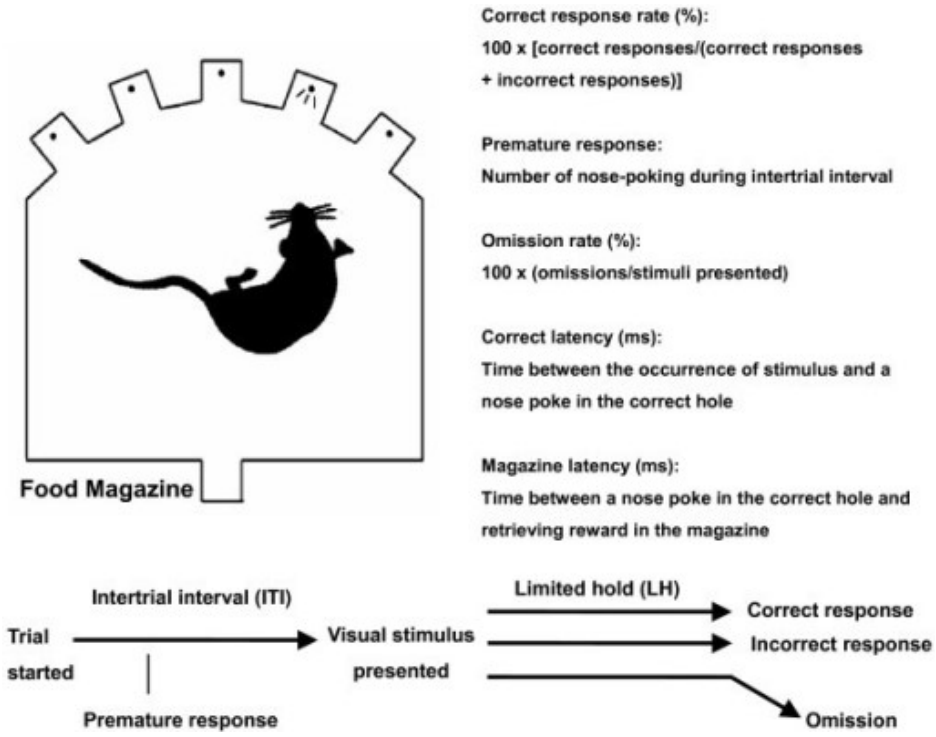


Figura 10.- *Esquema del procediment que es fa servir per avaluar diferents funcions cognitives al 5-CSRTT. Un assaig comença amb un interval de temps en el que l'animal pot fer una resposta prematura si introdueix el morro a un dels 5 forats. Quan s'acaba l'interval i es presenta un estímul (un llum) que senyala on ha d'anar l'animal per tal de rebre el reforç a la menjadora (food magazine). L'animal pot fer tres tipus de resposta: 1) introduir el morro al forat que s'havia senyalitzat amb el llum (resposta correcta, 2) acostar-se a una altra zona (resposta incorrecta) ó 3) no fer res (omissió). Extret de Liu et al.(J Biomed Sci. 16:72, 2009)*

4.4 Models farmacològics de sistemes neuroquímics específics: Dopamina

Els agonistes dopaminèrgics més utilitzats per modelar els símptomes de l'esquizofrènia en animals han sigut l'amfetamina i l'apomorfina. En models animals aquestes substàncies (agonistes dopaminèrgics) indueixen estereotípies motores, hiperactivitat i dèficits de PPI. Per exemple l'administració repetida d'amfetamina provoca sensibilització de la resposta locomotora (augment de l'activitat motora per l'administració repetida i intermitent de la substància), que s'ha postulat com un model vàlid dels símptomes positius de l'esquizofrènia, ja que segons alguns autors comparteix mecanismes neurobiològics. Aquest procés s'ha relacionat amb la regulació a la baixa dels receptors D3, alteracions de la neurotransmissió dopaminèrgica al nucli accumbens i un augment de la dimerització dels receptors D2 (Steeds et al., 2015). D'aquesta manera, aquests models si que mostren un cert grau de validesa aparent (i potser validesa de constructe) respecte als símptomes positius. Respecte a la validesa predictiva s'ha observat que els antipsicòtics, ja siguin els típics com els atípics, poden revertir els dèficits de PPI provocats pels agonistes dopaminèrgics com l'apomorfina (Geyer et al., 2001).

Segons Marcotte i col·laboradors (2001) la validesa de constructe d'aquests models és limitada ja que no hi ha suficients evidències científiques en les que el sistema dopaminèrgic tingui un paper principal en el desenvolupament del trastorn. No obstant, respecte als dèficits de PPI la DA té un paper important. Per exemple, els primers estudis de Mansbach i col·laboradors (1988) van mostrar que l'apomorfina provoca una disminució de la PPI i que l'haloperidol revertia aquest dèficit.

Aquestes alteracions, tant de la PPI com de l'activitat motora, estarien provocades per l'augment de DA al sistema límbic, en el cas de l'amfetamina pel boqueix del transportador de DA i per una disrupció de les vesícules sinàptiques que provocarien un augment de la DA a l'espai sinàptic. Per altra banda, l'apomorfina faria els seus efectes directament sobre els receptors de dopamina D1 i D2 (Geyer et al., 2001; McLean, 2010). En general, els models farmacològics basats en la DA provoquen alteracions relacionades amb els símptomes positius del trastorn, però no amb els símptomes negatius ni els cognitius. Per tant, aquesta mancança a l'hora de modelar els altres tipus de símptomes evidencia un grau de validesa de constructe baix.

4.5 Models farmacològics de sistemes neuroquímics específics: Serotonina

Els models animals en que s'indueixen els símptomes mitjançant l'administració d'agonistes serotoninèrgics produeixen un rang de símptomes més gran que en els models animals basats en la DA. Segons González-Maeso i Sealfon (2009), els agonistes serotoninèrgics indueixen símptomes característics de l'esquizofrènia paranoide.

Alguns dels fenotips generats per l'administració d'agonistes 5-HT2A, com ara el DOI o el LSD (2,5-Dimetoxi-4-iodoamfetamina i dietilamida de l'àcid lisèrgic, respectivament), són els dèficits de l'habituació de la resposta de sobresalt, dèficits de PPI i dèficits en la inhibició latent. Per això els models serotoninèrgics basen la seva validesa de constructe en la hipòtesi que, tant els símptomes esquizofrènics com els dèficits produïts per aquestes substàncies, estan provocats per respostes exagerades davant d'estímuls sensorials/cognitius que serien el resultat dels dèficits de filtratge atencional.

No obstant hi ha dos factors que disminueixen la validesa d'aquests models quan es comparen els resultats en humans amb els dels rosegadors: un és la rapidesa en que les substàncies com l'LSD generen tolerància, i el segon és el fet que produeixen al·lucinacions visuals, mentre que en l'esquizofrènia les al·lucinacions auditives són les més freqüents (Geyer i Vollenweider, 2008)

4.6 Models farmacològics de sistemes neuroquímics específics: Glutamat

Per la seva banda, els models animals farmacològics basats en la neurotransmissió glutamatèrgica si que són capaços d'induïr un ventall de dèficits i símptomes més ampli que els models basats en la DA i la 5-HT. Per exemple, segons González-Maeso i Sealfon (2009) aquests models es caracteritzen per la inducció de simptomatologia negativa i catatònica.

Els models farmacològics que tenen com a diana molecular els receptors N-metil-d-aspartat (NMDA) parteixen de l'observació, als anys 1950s, que la fenciclidina (PCP, Sernylan® Parke Davis; Luby et al., 1959) produïa símptomes psicòtics en humans. La PCP és una substància que administrada de forma aguda a les persones provoca al·lucinacions, deliris, trastorns del pensament i dèficits cognitius (Neill et al., 2010).

Per Steeds et al. (2015) els models que fan servir els antagonistes NMDAR tenen l'avantatge de reproduir els símptomes negatius i cognitius, i això fa que siguin adequats per tal de descobrir noves teràpies per revertir aquest tipus de símptomes, que afecten molt especialment a la vida social de les persones amb el trastorn.

Diferents estudis que han avaluat tant els efectes dels agonistes serotoninèrgics com dels antagonistes NMDAR han vist que hi ha interaccions funcionals entre els dos sistemes. S'ha vist que l'administració d'antagonistes no-competitius NMDAR provoquen un augment de la 5-HT extracel·lular, així com del metabòlit 5-HIAA (àcid 5-hidroxindolacètic) al CPF (Aghajanian i Marek, 2000). Altres estudis també han observat que els antagonistes 5-HT2 son capaços de revertir els dèficits que produeixen els antagonistes no competitius NMDAR (Aghajanian i Marek, 2000; Fribourg et al., 2011). A part d'aquestes interaccions, González-Maeso i colaboradors (2007 i 2008) han aportat dades sobre la interacció entre els receptors 5-HT2A i mGlu2 (Figura 11), i la seva funció en fenotips com el *head-twitch* o la PPI, que són molt rellevants per tal de desenvolupar models animals de l'esquizofrènia (veure Figura 11; González-Maeso et al., 2007).

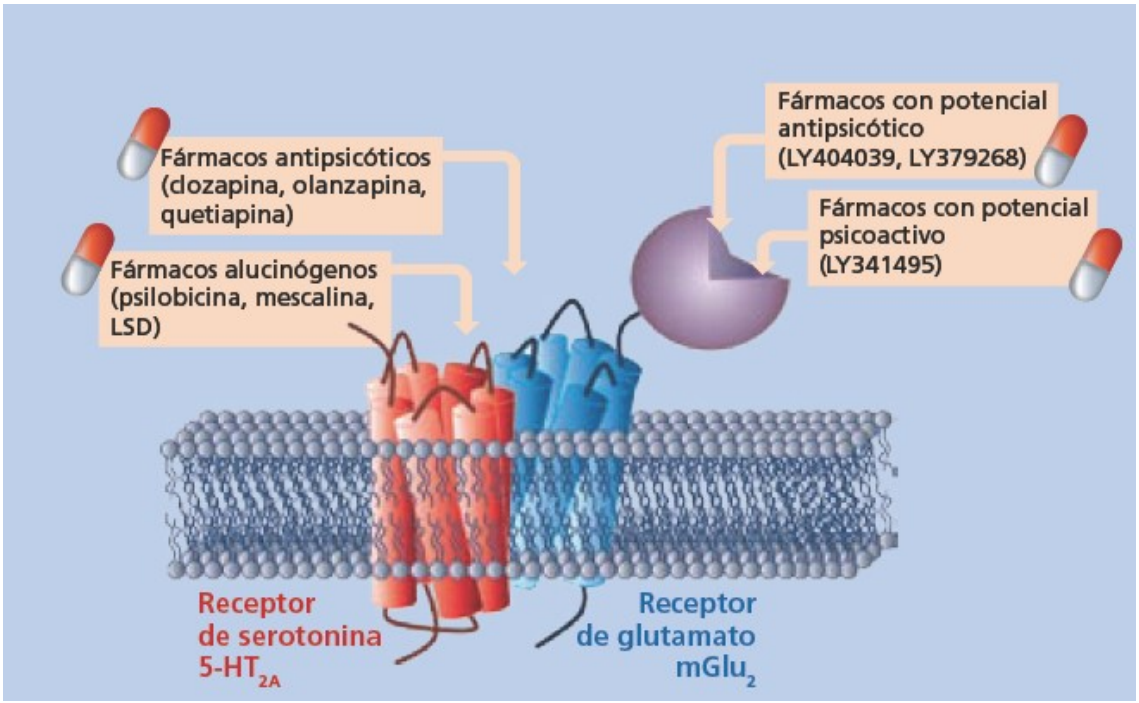


Figura 11.- *Imatge que mostra la interacció entre els 2 receptors que formen l'heterocomplex 2AR-mGluR2, on actuen les substàncies propsicòtiques i antipsicòtiques per induir els seus efectes. Extret de Moreno et al.,(Mente y cerebro, 44: 18-27, 2010a).*

4.7 Models del neurodesenvolupament

Els models del neurodesenvolupament s'han centrat en diverses manipulacions/estressors, administrades durant els períodes pre, peri i postnatal, que en humans s'ha vist que augmenten el risc a patir esquizofrènia (vegeu Figura 12). Per exemple, l'administració del virus de la *influenza* en ratolins al dia 9 de l'embaràs, o altres manipulacions que activen el sistema immunològic de les femelles embarassades, com ara l'exposició a l'endotoxina bacteriana lipopolisacarid (LPS), a l'àcid poliriboinosínic:poliribocitidíl·lic (Poly I:C) o al metilazoximetanol al dia gestacional 15, que provoquen canvis bioquímics i conductuals en rosegadors semblants als que es troben en pacients esquizofrènics (Fatemi et al., 2005; Jones et al., 2011; Meyer i Feldon, 2009, 2010). Complicacions al naixement, com poden ser la cesària o la hipòxia també provoquen canvis, tant pel que fa a la resposta a l'estrès com a la neurotransmissió dopaminèrgica en diferents espècies (Boksa i El-Khodor, 2003; Vaillancourt i Boksa, 2000; Wakuda et al., 2008). La lesió de l'hipocamp ventral al dia postnatal 7 també ha servit per modelar algunes de les característiques de l'esquizofrènia, com els dèficits cognitius o de PPI, i la hiperactivitat provocada pels psicoestimulants (Tseng et al., 2009). L'estrès prenatal també provoca una disminució del volum de l'hipocamp i dèficits cognitius, a més a més d'alteracions en els sistemes serotoninèrgics i dopaminèrgics (Koenig et al., 2002). La deprivació maternal constitueix un altre model, amb el que, per exemple, Ellenbroek et al. (1998) van mostrar que la separació maternal de 24 h produïa dèficits de PPI als 69 dies de vida i que aquests dèficits es podien revertir amb haloperidol i quetiapina. En aquest sentit, l'aïllament social a partir del dia 21 de vida també ha estat un model animal molt estudiat, ja que produeix efectes en l'expressió gènica, bioquímics i conductuals que s'assemblen als que es troben en pacients amb el trastorn (Fone i Porkess 2008; Powell i Geyer 2002).

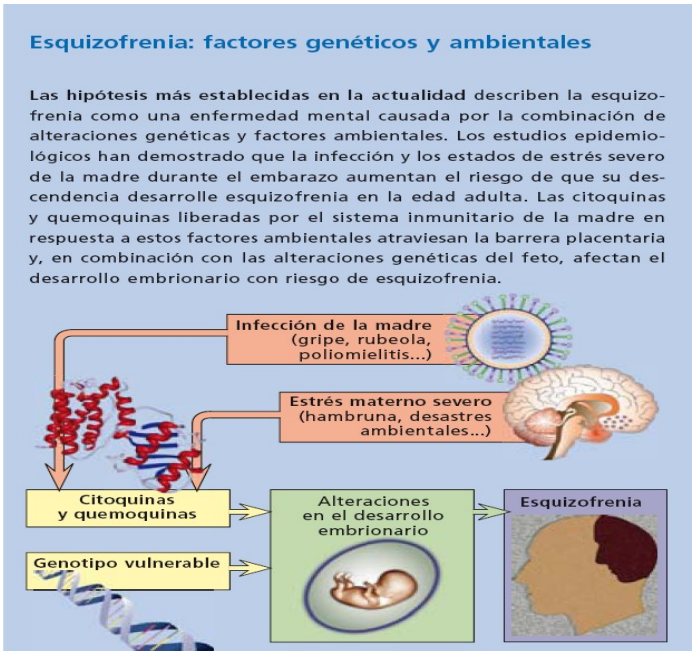


Figura 12.- *Factors genètics i ambientals del període pre i perinatal que augmenten el risc de patir esquizofrènia. Extret de Moreno et al. (Mente y cerebro, 44: 18-27, 2010a)*

L'aïllament social (AS) permet observar i avaluar l'efecte de l'estrès ambiental sobre el sistema nerviós, i per tant té l'avantatge de no requerir cap manipulació aguda com passa en altres models com els farmacològics. Usualment l'AS comença a la tercera setmana de vida dels animals (després del deslletament) i acaba a les 9-12 setmanes. Aquest procediment provoca canvis molt importants, ja que les rates estableixen una jerarquia social dins de la gàbia que té un impacte molt gran en el desenvolupament cerebral. Per aquest motiu l'AS i l'estrès que provoca produeixen molts canvis tant a nivell estructural com molecular del SNC i, conseqüentment, comporten uns canvis conductuals que tenen certes similituds amb els símptomes i característiques que trobem en pacients esquizofrènics (Fone i Porkess, 2008; Lapiz et al., 2003).

Els canvis conductuals produïts per l'AS reben el nom d'*isolation syndrome,* i inclouen els dèficits en PPI, la hiperactivitat, la reducció de les interaccions socials, un augment de l'agressivitat i dèficits de memòria i d'aprenentatge (per una revisió veure Fone i Porkess, 2008). A més a més, també s'ha associat l'AS amb la hiperactivitat dopaminèrgica a la via mesolímbica, hipoactivitat dopaminèrgica a la via mesocortical, a la reducció del volum del CPF i a la reducció de la plasticitat neuronal a l'hipocamp, canvis que també s'observen en pacients esquizofrènics (Fone i Porkess, 2008). L'avantatge d'aquest model

és que reprodueix una varietat molt gran dels fenotips associats a l'esquizofrènia, a més a més que també té una certa validesa predictiva, ja que els antipsicòtics reverteixen els efectes de l'AS. El model també presenta validesa de constructe, ja que els efectes neurobiològics que indueix són similars als que apareixen en pacients esquizofrènics. Per la seva banda, els desavantatges del model es refereixen als requeriments d'espai per estabular els animals de forma individual, així com en el fet que l'AS no és un procediment discret i per tant no es pot concretar en quin moment es produeixen els efectes.

L'AS també provoca altres efectes neuroquímics, com l'augment del receptor 5-HT1A a l'hipocamp (Del-Bel et al., 2002; Preece et al., 2004). La sinaptofisina una proteïna relacionada amb l'alliberació de neurotransmissor a les sinapsis, està disminuïda en algunes regions de l'hipocamp en animals criats en AS, i també s'han trobat reduccions de BDNF i un decrement de la densitat d'espines dendrítiques (Powell et al., 2010)

Per tant l'AS aporta un alt grau de validesa predictiva en els experiments amb antipsicòtics, i els diferents estudis conductuals han aportat evidències que fan d'aquest un model rellevant per l'avenç de la recerca en diferents trastorns mentals en els que el desenvolupament del sistema nerviós té un paper molt important.

4.8 Models genètics

A partir dels estudis de bessons s'ha calculat que l'heretabilitat de l'esquizofrènia podria arribar a un 80%, és a dir, els factors genètics semblen tenir molta importància en l'etiologia del trastorn (Elert, 2014; Harrison i Owen, 2003). A partir d'aquestes dades, molts models animals s'han centrat en estudiar quin rol tenen diferents gens en diferents fenotips. Aquests models animals són sobretot els ratolins knock-out (KO), als que se'ls hi ha eliminat un gen. Molts dels gens pels que s'han creat models animals KO, són gens que sintetitzen proteïnes que estan relacionades amb la plasticitat neuronal i els sistemes glutamatèrgic i dopaminèrgic (Jones et al., 2011).

Tenint en compte l'alt grau d'homologia entre els gens dels ratolins i dels humans, l'estudi de ratolins KO (de gens susceptibles de ser responsables en part de l'etiologia de l'esquizofrènia) ha permès desenvolupar models animals amb un cert grau de validesa. En el cas de les rates la tecnologia que permet la manipulació de gens ha progressat més

lentament, i els primers estudis amb rates amb manipulacions genètiques, per tal de modelar alguns dels trets neuroquímics de l'esquizofrènia, van aparèixer fa uns 10 anys (Ellenbroek i Karl, 2016). Les rates knock-out pel transportador de 5-HT i la rata hipomòrfica per la neuroregulina 1, que mostren algunes característiques neuroquímiques i fenotips semblants al que mostren els pacients esquizofrènics (Homberg et al., 2007; Taylor et al., 2011), són dos exemples.

En paral·lel, s'han desenvolupat altres estratègies per tal de generar models animals vàlids. Una d'aquestes estratègies és la selecció psicogenètica, en la que les rates que presenten una característica o fenotip concret es reprodueixen entre elles. Tot i que l'estratègia de seleccionar les rates per una conducta específica pot fer pensar que aquesta estratègia sigui errònia, ja que els trastorns neuropsiquiàtrics no acostumen a tenir símptomes patognòmics (que els diferencien dels altres trastorns), hi ha molts exemples de soques de rates que han servit per modelar diferents trastorns o símptomes (Ellenbroek i Karl, 2016). Per exemple, les rates sensibles/resistents als inhibidors de l'acetilcolinesterasa que van ser seleccionades segons la seva resposta a aquestes substàncies, i que actualment són considerades un model animal de depressió, doncs presenten molts dels símptomes i biomarcadors del trastorn (Knapp et al., 2014).

En el cas de l'esquizofrènia, trobem diferents soques de rates que han estat creades seguint una estratègia semblant. Per exemple, les rates susceptibles (i les no susceptibles) als efectes de l'apomorfina (APO-SUS i APO-UNSUS) que han estat seleccionades per la quantitat/intensitat d'estereotípies que mostren quan se'ls hi administra apomorfina (Selten et al., 2016). Les rates Brattleboro, que van ser seleccionades perquè una cohort de rates Long Evans tenia una mutació que provocava que la quantitat de vasopresina alliberada per aquestes rates fos molt petita. A causa d'això, aquestes rates presenten polidípsia i poliúria, i es consideren un model animal de la *diabetes insipidus* (Del Río et al., 2014; Ellenbroek i Karl, 2016), però també presenten alguns trets o símptomes que modelen certs aspectes de l'esquizofrènia (Cilia et al., 2010). Per altra banda, les rates seleccionades per el seus valors de pressió arterial presenten moltes de les característiques dels pacients amb trastorn per dèficit d'atenció i hiperactivitat, i també presenten certes característiques que s'assemblen a les dels pacients esquizofrènics (Meneses et al., 2011). Totes aquestes soques de rates van ser seleccionades per criteris o trets inicialment independents dels fenotips relacionats amb l'esquizofrènia, però posteriorment s'ha vist que presenten alguns trets o símptomes relacionats amb aquest trastorn. Finalment, també

existeixen rates seleccionades per els seus nivells extrems (alts o baixos) de PPI, fenotip que com ja hem dit representa funcions sensorimotores (filtratge atencional) que estan deteriorades en els pacients esquizofrènics (Hadamitzky et al., 2007; Schwabe et al., 2007).

En definitiva, molts dels models genètics esmentats presenten graus rellevants de validesa aparent, i alguns d'ells inclús certa validesa predictiva, doncs s'han testat els efectes de fàrmacs antipsicòtics (p. ex. Hadamitzky et al., 2007) i drogues psicoestimulants (vegeu revisió de Del Río et al., 2014).

5. Les rates *Roman* d'alta evitació (RHA), respecte de les *Roman* de baixa evitació (RLA), com a model d'alguns símptomes de l'esquizofrènia

Les rates Romanes deriven de rates Wistar (*Rattus Norvegicus*), que han estat seleccionades per la seva habilitat per adquirir l'aprenentatge de l'evitació activa en dos sentits. D'aquesta manera, podem distingir entre les rates Romanes d'alta evitació (RHA), que aprenen amb molta facilitat la tasca, i les rates Romanes de baixa evitació (RLA), que pràcticament no són capaces d'aprendre la tasca. Aquestes soques, que es van crear l'any 1965 (Bignami, 1965), han estat mantingudes a Suïssa (des del 1972) sota un règim de creuament selectiu (i no consanguini) per tal de mantenir l'estabilitat fenotípica, i han estat àmpliament estudiades en diferents publicacions (p. ex. Steimer i Driscoll, 2005). A part de la colònia de rates de Suïssa, anomenades RHA/Verh i RLA/Verh, una altra colònia de rates no consanguínies (que també deriva de la de Suïssa) es troba a Cagliari, Italia (Giorgi et al., 2007). A l'any 1993, a partir de la colònia de rates de Suïssa es va iniciar un programa de creuament per desenvolupar soques de rates Romanes consanguínies (RHA-I i RLA-I), que es mantenen a la Unitat de Psicologia Mèdica del Departament de Psiquiatria i Medicina Legal de la Universitat Autònoma de Barcelona (Driscoll et al., 2009; Escorihuela et al., 1999).

Entre les diferències fenotípiques, que hi ha entre les línies/soques RHA i RLA, destaquen les diferències en reactivitat emocional, ansietat i por. Concretament, les rates RLA presenten més reactivitat emocional, ansietat i por davant de situacions de novetat, de conflicte (condicionat o incondicionat) i/o d'estrès moderat (Driscoll et al. 2009;

Fernández-Teruel et al., 1997). Les rates RLA també presenten una resposta hormonal augmentada davant l'estrès: nivells d'hormona adrenocorticotropa (ACTH), corticosterona i prolactina augmentats respecte les RHA (p. ex. Carrasco et al. 2008; Díaz-Morán et al. 2012; Steimer et al., 1997, Steimer i Driscoll, 2003). En definitiva, les RHA presenten un perfil d'afrontament amb baixa ansietat i proactiu (p. ex. Díaz-Morán et al. 2012; Piras et al. 2010; Steimer et al. 1997; Steimer i Driscoll 2003), que s'evidencia pel seu perfil de conducta activa en situacions de conflicte/estrès, de conducta impulsiva i de percaça de sensacions (vegeu revisió de Driscoll et al., 2009). Per exemple, Moreno et al. (2010b) van observar que les rates RHA mostren més conducta impulsiva i més errors a les tasques de *"delay dicounting"* i al 5-CSRTT (Klein et al. 2014; Moreno et al., 2010b). En general, les rates RHA mostren un comportament més actiu, impulsiu, inquisitiu i agressiu. També mostren una preferència major per drogues, i tenen una resposta motora més gran que les RLA després de l'administració aguda i/o repetida dels agonistes dopaminèrgics (Fattore et al. 2009; Giménez-Llort et al. 2005; Giorgi et al. 2007; Guitart-Masip et al., 2008a; Manzo et al. 2014b; Tournier et al. 2013).

Per l'altra banda, les RLA presenten un tipus de perfil d'afrontament reactiu i d'alta ansietat, i per tant, presenten un augment de les conductes ansioses i de por. Segons Driscoll et al. (2009) les rates RLA executen millor les tasques que no involucren un estrès agut excessiu (p. ex. un xoc elèctric), com poden ser tasques de memòria i aprenentatge espacial, discriminació d'objectes o prémer una palanca per aconseguir un reforç (Aguilar et al., 2002; Driscoll i Bättig, 1982; Driscoll et al., 1995; Fernández-Teruel et al., 1997; Nil i Bättig, 1981; Zeier et al., 1978). Per tant, Driscoll et al. (2009) conclouen que la millor execució de les rates RHA a l'evitació activa en dos sentits a la *Shuttle box* no es deu a una millora de les habilitats en l'aprenentatge de les rates RHA, sinó, a factors emocionals, i a les estratègies d'afrontament que diferencien les dues soques.

Per tal d'explicar les diferències fenotípiques, el sistema més explorat en aquestes soques de rates ha estat la dopamina (DA). Una de les diferències, que podria explicar les divergències fenotípiques entre les RHA i les RLA és que, en les primeres hi ha un augment d'alliberació de DA al CPF en situacions d'estrès (D'Angio et al., 1988, Giorgi et al., 2003). Un altre estudi, de Tournier et al. (2013), va demostrar que les rates RHA tenen disminuït l'autoreceptor D2 de la via nigroestriada. Això provocaria un augment de DA a l'estriat, ja que els auto-receptors D2 inhibeixen l'alliberació de DA a aquesta àrea.

Tournier i colaboradors (2013) també van observar que les rates RHA presentaven nivells més elevats de Tirosina Hidroxilasa (TH), més DA extracel·lular a l'estriat i més alliberació de DA quan s'administra amfetamina. Una altra dada consistent amb els resultats de Tournier et al. (2013), és a l'estudi d' Eilam i Szechtman (1989), que van veure que les rates RHA mostraven menys sensibilitat a la quinpirola (un antagonista dels receptors D2). Segons Tournier et al. (2013) els efectes d'hipoactivitat provocats per aquest fàrmac estan mediats per els mateixos receptors D2 (tant presinàptics com postsinàptics), i aquests autors van veure com la inhibició de l'activitat motora només s'apreciava en les RLA (Eilam i Szechtman, 1989).

A l'estudi de Klein et al. (2014) també s'hi mostren altres diferències neuroquímiques de les dues soques. Concretament, van trobar que les RHA mostren nivells d'expressió més alts dels receptors de serotonina 5-HT1A, 5-HT2A i del transportador (5-HTT) al còrtex frontal. Per el contrari, els receptors de glutamat mGlu2 i mGlu3 estaven disminuïts en les RHA. Klein et al. (2014) també van observar que les rates RHA no mostraven nivells detectables de mGluR2 al còrtex frontal, l'hipocamp i l'estriat. Per Klein et al. (2014), aquestes diferències als sistemes serotoninèrgics i glutamatèrgics també tenen un paper important per explicar les diferències en impulsivitat i la vulnerabilitat a l'addició de les rates RHA.

Respecte a les característiques neuroquímiques, que podrien donar validesa a les rates RHA com a model de símptomes rellevants per l'esquizofrènia, els resultats de Klein et al. (2014) mostrant que les rates RHA presenten nivells augmentats de receptors 5-HT2A i disminuïts de mGlu2 al CPF, són coincidents amb el que va trobar González-Maeso (2008) en pacients esquizofrènics no tractats. Aquest patró, tal com senyalen González-Maeso (2008) i Wischhof i Koch (2016), és essencial pels processos de filtratge atencional i podria augmentar la vulnerabilitat als trastorns psicòtics (Wischhof i Koch, 2016).

Juntament amb les diferències RHA vs RLA al sistema dopaminèrgic i al complex 2AR-mGluR2, el fet que les RHA siguin més susceptibles de ser sensibilitzades (sensibilització locomotora) per psicoestimulants (Corda et al., 2005; Giménez-Llort et al., 2005; Giorgi et al., 2007; Guitart-Masip et al., 2008a), i presentin altres diferències fenotípiques com la hiperactivitat locomotora espontània (Steimer i Driscoll, 2003), els dèficits d'inhibició latent (Fernández-Teruel et al., 2006; Esnal et al., 2016), així com els dèficits de PPI

mostrats per primera vegada per Del Río et al. (2014), ens van fer pensar que les rates RHA podrien ser un model vàlid per alguns símptomes esquizofrènics.

6. Les rates genèticament heterogènies *"National Institutes of Health N/Nih Genetically Heterogeneous Rat stock"* (NIH-HS)

Per tal de poder desenvolupar un model animal que presenti característiques rellevants d'un trastorn determinat, és important conèixer també com es comporten aquestes característiques en la població general. En aquest sentit, les rates genèticament heterogènies (NIH-HS) són una soca que podria representar a la població general de rates. Les rates genèticament heterogènies *"National Institutes of Health N/Nih Genetically Heterogeneous Rat stock"* (NIH-HS) van ser desenvolupades per Hansen i Spuhler (1984) als "*National Institutes of Health* (NIH)" a partir de 8 soques de rates consanguínies amb el màxim possible de distància genètica entre elles (vegeu Figura 13). Aquestes 8 soques de rates eren la MR/N,WN/N y WKY/N (aquestes tres tenen com a ancestre comú l'estoc de rates Wistar), les M520/N i F344/N (creades a la dècada dels 20 del segle passat però d'origen desconegut), les ACI/N (un híbrid entre les soques d'August i de Copenhaguen), les BN/SsN (que deriven d'un mutant en el color de rates salvatges -*wild type*- de l'estoc del Wistar Institute) i les BUF/N (vegeu Hansen i Spuhler 1984). A continuació, aquestes 8 soques de rates es van creuar i criar durant més de 80 generacions, seguint un regim rotacional i no consanguini per tal d'evitar els efectes de la consanguinitat anterior, de la fixació i de la deriva genètica (p. ex. Alam et al., 2011; Baud et al., 2013; Johannesson et al., 2009). El factor més important de les rates NIH-HS és que cadascuna d'elles representa un mosaic genètic únic i aleatori dels cromosomes dels animals fundadors, gràcies a les recombinacions acumulades al llarg de tantes generacions (Figura 1). És aquest el motiu pel qual s'ha demostrat que aquests animals són una eina única per a l'estudi de les bases genètiques de tot tipus de trets o caràcters/fenotips complexos i quantitatius, inclosos els conductuals/psiquiàtrics (Alam et al., 2011, 2014; Baud et al., 2013, 2014a-b; Díaz-Morán et al., 2012, 2013a-c; Johannesson et al., 2009; López-Aumatell et al., 2008, 2009b, 2011). A més a més, les rates NIH-HS són una eina molt útil per tal d'establir relacions entre fenotips i genotips (Baud et al., 2013)

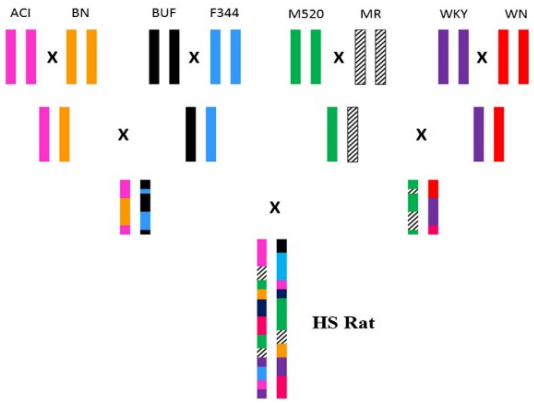


Figura 13.- *Creuament de les 8 soques de rates que van donar com a resultat les rates NIH-HS. Extret d' Alam et al. (Bone, 48*(5), 1169–1177*, 2011).*

La primera caracterització conductual que es va fer de les rates NIH-HS va consistir en fenotipar aquestes rates per 16 tipus de conductes relacionades amb la ingesta i sensibilitat a l'alcohol (Spuhler i Deitrich, 1984). En aquest estudi es va poder observar que els valors obtinguts de les rates NIH-HS estaven al mig dels valors extrems obtinguts per les soques de rates consanguínies parentals (les que es van fer servir per obtenir les rates genèticament heterogènies).

Pel que fa a altres fenotips o trets que caracteritzen aquest stock de rates heterogènies, s'ha vist que en tests que mesuren ansietat/por les rates NIH-HS presenten valors similars a les RLA o valors que es troben entre les dues soques de rates Romanes. En general en els tests que s'han realitzat al nostre laboratori, que s'han centrat en fenotips relacionats amb l'ansietat i la por, hem vist que les rates NIH-HS presenten nivells d'ansietat semblants a les rates RLA i un estil d'afrontament reactiu/passiu com les RLA. Aquest perfil d'afrontament, caracteritzat per la immobilitat davant situacions de conflicte o estrès, es troba present fins i tot en nivells més elevats que en les rates RLA, com es veu en tests com en el test de Porsolt (també a nomenat test de natació forçada), en que els nivells d'immobilitat són més alts i els d' intents de fugida són més baixos en les rates NIH-HS que en les RLA (Díaz-Morán et al., 2012).

Respecte a les funcions cognitives, Martinez-Membrives et al. (2015) van observar que l'execució de les rates NIH-HS és molt bona, i molt semblant a la de les rates RLA, en diferents tasques d'aprenentatge espacial i de memòria espacial a llarg termini realitzades al laberint aquàtic de Morris (Martinez-Membrives et al., 2015). En aquesta Tesi s'ha ampliat la caracterització conductual amb diferents fenotips relacionats amb símptomes

presents en trastorns mentals com l'esquizofrènia, com els dèficits de PPI i de memòria de treball (veure Estudi 1).

7. Objectius generals

1) Caracterització conductual i dels receptors 5-HT1A i 5-HT2A de les rates RHA-I i RLA-I, comparades amb les rates genèticament heterogènies NIH-HS.

2) Avaluar els efectes que té l'aïllament social sobre els dèficits associats amb l'esquizofrènia en les rates RHA-I/RLA-I.

3) Fer la caracterització psicofarmacològica de les soques RHA-I/RLA-I com a model animal genètic de vulnerabilitat diferencial a simptomatologia esquizofrènica.

8. Objectius específics

1) A través de la inhibició de la resposta de sobresalt mitjançant un prepols (PPI), i la tasca d'aparellament retardat a una posició al laberint aquàtic de Morris, avaluar dos processos que en pacients esquizofrènics estan alterats, la PPI i la memòria de treball.

2) Valorar els efectes que té l'aïllament sobre les soques de rates Romanes en diferents tasques que valoren el filtratge atencional (PPI), l'activitat motora (test d'activitat motora), l'ansietat (*elevated zero-maze*) i l'aprenentatge/memòria espacial (aparellament retardat a una posició i el test de transferència –memòria de referència-).

3) Avaluar a través de la PPI i l'activitat motora si les substàncies amb característiques propsicòtiqes i antipsicòtiques produeixen els efectes esperats a la soques Romanes en aquests dos tests.

4) Comprovar si el patró de nivells de receptors que es va observar a l'estudi de Klein i col·laboradors es veu reflectit en l'expressió gènica dels receptors de serotonina 1A i 2A mesurada a través de *Reverse transcription quantitative polymerase chain reaction* (RT-qPCR).

II. Resultats

9. ESTUDI 1:

Prepulse inhibition predicts spatial working memory performance in the inbred Roman high- and low-avoidance rats and in genetically heterogeneous NIH-HS rats: relevance for studying pre-attentive and cognitive anomalies in schizophrenia

frontiers in Behavioral Neuroscience

ORIGINAL RESEARCH
published: 18 August 2015
doi: 10.3389/fnbeh.2015.00213

Prepulse inhibition predicts spatial working memory performance in the inbred Roman high- and low-avoidance rats and in genetically heterogeneous NIH-HS rats: relevance for studying pre-attentive and cognitive anomalies in schizophrenia

OPEN ACCESS

Edited by:
Dorota Frydecka,
Wroclaw Medical University, Poland

Reviewed by:
Rutsuko Ito,
University of Toronto, Canada
Aleksei Yurievich Egorov,
Russian Academy of Sciences, Russia

***Correspondence:**
Ignasi Oliveras and Alberto Fernández-Teruel, Medical Psychology Unit, Department of Psychiatry and Forensic Medicine, School of Medicine, Autonomous University of Barcelona, 08913 Bellaterra, Barcelona, Spain
ignasi.oliveras@uab.cat; albert.fernandez.teruel@uab.cat

Received: *07 May 2015*
Accepted: *30 July 2015*
Published: *18 August 2015*

Citation:
Oliveras I, Río-Álamos C, Cañete T, Blázquez G, Martínez-Membrives E, Giorgi O, Corda MG, Tobeña A and Fernández-Teruel A (2015) Prepulse inhibition predicts spatial working memory performance in the inbred Roman high- and low-avoidance rats and in genetically heterogeneous NIH-HS rats: relevance for studying pre-attentive and cognitive anomalies in schizophrenia. Front. Behav. Neurosci. 9:213. doi: 10.3389/fnbeh.2015.00213

Ignasi Oliveras*[1]*, *Cristóbal Río-Álamos*[1], *Toni Cañete*[1], *Gloria Blázquez*[1], *Esther Martínez-Membrives*[1], *Osvaldo Giorgi*[2], *Maria G. Corda*[2], *Adolf Tobeña*[1] *and Alberto Fernández-Teruel*[1]

[1] Medical Psychology Unit, Department of Psychiatry and Forensic Medicine, School of Medicine, Institute of Neurosciences, Autonomous University of Barcelona, Barcelona, Spain, [2] Section of Pharmaceutical, Pharmacological and Nutraceutical Sciences, Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy

Animal models of schizophrenia-relevant symptoms are increasingly important for progress in our understanding of the neurobiological basis of the disorder and for discovering novel and more specific treatments. Prepulse inhibition (PPI) and working memory, which are impaired in schizophrenic patients, are among the symptoms/processes modeled in those animal analogs. We have evaluated whether a genetically-selected rat model, the Roman high-avoidance inbred strain (RHA-I), displays PPI deficits as compared with its Roman low-avoidance (RLA-I) counterpart and the genetically heterogeneous NIH-HS rat stock. We have investigated whether PPI deficits predict spatial working memory impairments (in the Morris water maze; MWM) in these three rat types (Experiment 1), as well as in a separate sample of NIH-HS rats stratified according to their extreme (High, Medium, Low) PPI scores (Experiment 2). The results from Experiment 1 show that RHA-I rats display PPI and spatial working memory deficits compared to both RLA-I and NIH-HS rats. Likewise, in Experiment 2, "Low-PPI" NIH-HS rats present significantly impaired working memory with respect to "Medium-PPI" and "High-PPI" NIH-HS subgroups. Further support to these results comes from correlational, factorial, and multiple regression analyses, which reveal that PPI is positively associated with spatial working memory performance. Conversely, cued learning in the MWM was not associated with PPI. Thus, using genetically-selected and genetically heterogeneous rats, the present study shows, for the first time, that PPI is a positive predictor of performance in a spatial working memory task. These results may have translational value for schizophrenia symptom research in humans, as they suggest that either

by psychogenetic selection or by focusing on extreme PPI scores from a genetically heterogeneous rat stock, it is possible to detect a useful (perhaps "at risk") phenotype to study cognitive anomalies linked to schizophrenia.

Keywords: prepulse inhibition, spatial working memory, cognitive deficits, schizophrenia-relevant symptoms, schizophreniform rat model, Roman high-avoidance rats, Roman low-avoidance rats, genetically heterogeneous rats

Introduction

Schizophrenia symptoms are usually grouped in three categories: positive (hallucinations, delusions, and other thought disorders); negative (anhedonia, avolition, poverty of thought, and content of speech) and cognitive impairment (working memory and attention deterioration). Their complexity, diversity, and bizarreness preclude a full modeling of the entire constellation in animals, just as schizophrenic patients do not manifest every possible symptom. Some of the most commonly used animal models rely on the similarity between the effects that psychotomimetic and psychostimulant drugs trigger in both animals and humans, i.e., those animal analogs mostly reproduce positive (psychotic) symptoms of schizophrenia, while other rodent analogs focus on modeling negative (e.g., impaired social behavior) or cognitive (e.g., impairments of spatial learning, working memory) symptoms of the disorder. In addition, some rat and mouse models may be used to assess sensorimotor gating (pre-attentive) or attention-related processes which are impaired in schizophrenic patients (e.g., reviews by Sawa and Snyder, 2002; Powell and Miyakawa, 2006; Jones et al., 2011; Del Río et al., 2014).

One of these pre-attentive processes is prepulse inhibition (PPI), which refers to the ability of an acoustic stimulus of relatively weak intensity (i.e., prepulse) to diminish the acoustic startle response (ASR) caused by a subsequent acoustic pulse of higher intensity. PPI is an operational measure of the pre-attentive filtering process known as sensorimotor gating, which reflects the neural filtering of redundant or unnecessary stimuli that takes place in complex systems (Freedman et al., 1987; Koch and Schnitzler, 1997; Cromwell et al., 2008; García-Sánchez et al., 2011; Kohl et al., 2013). Since PPI is a cross-species phenomenon, it can be measured in both mammals and humans with the same experimental procedure, thereby providing a very useful paradigm for translational research. PPI is impaired in schizophrenic patients, among other mental disorders, and thus it is widely considered an endophenotype of the disorder (e.g., reviews by Freedman et al., 1987; Koch and Schnitzler, 1997; Cromwell et al., 2008; García-Sánchez et al., 2011; Kohl et al., 2013).

Genetically-based rat models of schizophrenia-related symptoms, derived from selective breeding programs, may have the advantage of symptom stability (within and across generations), and may lead to the identification of clusters of associated/related symptoms. Hence, genetic models represent a useful approach to study the neurobiological and, importantly, genetic mechanisms underlying the symptoms of schizophrenia. Examples of these genetically-based rat models are, for instance, the APO-SUS and APO-UNSUS rat lines (Ellenbroek et al., 1995; van der Elst et al., 2006, the "three hit" Low-PPI rat line (Petrovszki et al., 2013; Kekesi et al., 2015) and the Low-PPI/High-PPI rat lines (Freudenberg et al., 2007; Schwabe et al., 2007), which are rat lines presenting impairments in PPI (APO-SUS, "three hit low-PPI" and Low-PPI) and other schizophrenia-related symptoms, like latent inhibition or some cognitive functions (Ellenbroek et al., 1995; van der Elst et al., 2006; Freudenberg et al., 2007; Schwabe et al., 2007; Petrovszki et al., 2013; Kekesi et al., 2015; see review by Del Río et al., 2014).

The Roman High- (RHA) and Low-avoidance (RLA) rat lines/strains (depending on whether they are outbred –i.e., lines-, or inbred –i.e., strains-), may constitute another of such genetic models. They have been selectively and bidirectionally bred for their rapid (RHA) vs. extremely poor (RLA) ability to acquire the two-way active avoidance task (Bignami, 1965; Broadhurst and Bignami, 1965). The extensive research conducted over the last 40 years (e.g., Zeier et al., 1978; Driscoll and Bättig, 1982; Escorihuela et al., 1995a) has led to the conclusion that anxiety/fear and stress sensitivity are among the most prominent behavioral traits that distinguish the two Roman lines/strains. In fact, compared to their RLA counterparts, RHA rats (both from the outbred line –RHA/Verh- or from the inbred strain –RHA-I-) show a phenotype characterized by: (1) low unconditioned and conditioned anxiety/fear (López-Aumatell et al., 2009a,b,c; Díaz-Morán et al., 2012, 2013c; Martínez-Membrives et al., 2015), (2) a proactive coping style (Steimer and Driscoll, 2003, 2005; Driscoll et al., 2009; Díaz-Morán et al., 2012), (3) decreased sensitivity to reward-loss-induced frustration (e.g., Torres et al., 2005; Rosas et al., 2007; Gomez et al., 2009), (4) lowered activation of the hypothalamus–pituitary–adrenal (HPA) axis in response to stress (Steimer and Driscoll, 2003; Carrasco et al., 2008), (5) enhanced central GABA-A/benzodiazepine complex function (which is known to be critically involved in the regulation of anxiety/frustration; Corda et al., 1997; Bentareha et al., 1998) and (6) increased novelty- and drug-seeking behavior (e.g., Fernández-Teruel et al., 1992, 1997; Escorihuela et al., 1999; Giorgi et al., 2007; Manzo et al., 2014). Most important to assess whether RHA/RLA rats display differential schizophrenia-relevant features, the RHA strain/line displays a poorer performance in several learning/memory tasks (Nil and Bättig, 1981; Driscoll et al., 1995; Escorihuela et al., 1995b; Aguilar et al., 2002) and enhanced impulsive behavior in the 5-CSRTT and DRL-20 operant tasks (Zeier et al., 1978; Moreno et al., 2010; Klein et al., 2014). These profiles suggest that RHA rats may have some value for modeling certain deficits of executive function present in schizophrenia. Compared with the RLA line/strain and/or with standard rat

strains, RHA rats display relative deficits in latent inhibition threshold (Fernández-Teruel et al., 2006; and unpublished results from our laboratory), augmented mesocortical dopaminergic response to stress (Giorgi et al., 2003), enhanced locomotor as well as mesolimbic dopaminergic sensitization to repeated (DAergic) psychostimulant administration (Corda et al., 2005; Giorgi et al., 2007; Guitart-Masip et al., 2008), and neurochemical and neuromorphological evidence of decreased hippocampal function (Sallés et al., 2001; Meyza et al., 2009; Garcia-Falgueras et al., 2012). Remarkably, we have recently found that RHA-I rats show a dramatically reduced expression of mGluR2 in prefrontal cortex and hippocampus and increased cortical 5HT2AR expression (Klein et al., 2014). Thus, RHAs display a series of neurobehavioral traits that resemble some schizophrenia—relevant symptoms or associated neural processes.

As said above, schizophrenias usually present (or are associated with) complex clusters of symptoms. Knowing which of them are inter-related or which are orthogonal is important for both, progress in neurobiological research and for the development of novel treatments addressed to particular symptoms or clusters of symptoms. In this context, clinical researchers are studying the relationships among pre-attentive processes, attention, memory, cognition, and executive functions in schizophrenics and healthy human volunteers (Bitsios and Giakoumaki, 2005; Bitsios et al., 2006; Giakoumaki et al., 2008; Csomor et al., 2009). Using this approach it has been shown that PPI may be positively correlated with some cognitive functions, including working memory, in healthy human volunteers (Bitsios and Giakoumaki, 2005; Bitsios et al., 2006; Giakoumaki et al., 2008; Csomor et al., 2009; Singer et al., 2013). In a recent study in mice, Singer et al. have also shown associations between PPI and working memory (Singer et al., 2013).

In the present study we aimed at evaluating possible links between PPI and working memory in the genetically-selected inbred RHA-I and RLA-I rats (supposedly "altered" because of the psychogenetic selection, and therefore representing a parallel of a "clinical" or "at risk" sample), and in the genetically heterogeneous (i.e., outbred) NIH-HS rat stock as a plausible parallel of a normative and healthy human sample. The genetically heterogeneous NIH-HS rat stock (i.e., "National Institutes of Health Genetically Heterogeneous Rat Stock") was developed by Hansen and Spuhler (1984) through an eight-way cross from eight inbred rat strains and they were bred for more than 50 generations. The NIH-HS rats are a unique tool to study the genetic basis of complex traits due to their broad phenotypic variation and high degree of genetic recombination compared to the usual laboratory rat strains (e.g., Spuhler and Deitrich, 1984; López-Aumatell et al., 2008, 2009a,b, 2011; Johannesson et al., 2009; Vicens-Costa et al., 2011; Díaz-Morán et al., 2012, 2013a,b,c, 2014; Baud et al., 2013, 2014a,b; Estanislau et al., 2013; Palència et al., 2013; Alam et al., 2014; Tsaih et al., 2014). Moreover, NIH-HS rats have been shown to closely resemble RLA-I rats in their coping style and stress sensitivity profiles (e.g., López-Aumatell et al., 2009a; Díaz-Morán et al., 2012, 2013c; Estanislau et al., 2013). Taking into account these characteristics, we used the NIH-HS rats because of their potentially high translational value.

Therefore, the aim of this research was to investigate possible sensory gating-working memory relationships by: (1) characterizing the performance of the three rat strains/stocks (RHA-I, RLA-I, and NIH-HS) for PPI and spatial working memory in the delayed-matching-to-place in the Morris Water Maze (MWM); and (2) evaluating PPI-working memory associations in a sample of heterogeneous NIH-HS rats stratified by their extreme (low or high) PPI levels.

On the basis of the results reviewed above, we hypothesized that (1) RHA-I rats would show PPI and working memory deficits as compared to RLA-I and NIH-HS rats (note that, as said above, RLA-Is and NIH-HS have a very similar behavioral/neuroendocrine profiles in several tests/tasks), and (2) PPI levels would be positively associated with spatial working memory.

Materials and Methods

Animals

The animals used were males of the inbred Roman High- (RHA-I) and Low-Avoidance (RLA-I) rat strains and the genetic heterogeneous rat stock (NIH-HS, "National Institutes of Health Genetically Heterogeneous Rat Stock"; derived from crossing the MR/N, WN/N, WKY/N, M520/N, F344/N, ACI/N, BN/SsN, and BUF/N strains; Hansen and Spuhler, 1984), from the permanent colonies maintained at our laboratory (Medical Psychology Unit, Dept. Psychiatry, and Forensic Medicine, School of Medicine, Autonomous University of Barcelona) since 1996 (RHA-I, RLA-I) and 2004 (NIH-HS), respectively. They were approximately 4 months old at the beginning of the experiments (weight range 320–420 g), and were housed in same-sexed pairs in standard ($50 \times 25 \times 14$ cm) macrolon cages. They were maintained under a 12:12 h light-dark cycle (lights on at 08:00 a.m.), with controlled temperature ($22 \pm 2^{\circ}$C) and humidity (50–70%) and with free access to food and water.

In Experiment 1 subjects were male rats, from the RHA-I ($n = 16$) and RLA-I ($n = 19$) strains and from the NIH-HS genetically heterogeneous rat stock ($n = 30$), which were submitted to PPI testing and to the working memory tasks (see below). The same RHA-I and RLA-I rats were tested in the cued leaning task, jointly with 17 NIH-HS rats that were randomly selected from the initial sample of 30 animals.

In Experiment 2 subjects were 78 NIH-HS rats which were tested for PPI. From these, 33 NIH-HS rats were randomly selected to be evaluated in the working memory task. From these, 5–6 randomly selected rats from each of the three subgroups formed (see details below) underwent the cued learning task.

Rats in the RHA-I and RLA-I groups came from at least 10 different litters/strain, while NIH-HS rats were from 30 litters in each experiment.

The experiments were performed from 9:00 to 18:00 h. and were approved by the committee of Ethics of the Autonomous University of Barcelona in accordance with the European Communities Council Directive (86/609/EEC) regarding the care and use of animals for experimental procedures.

Prepulse Inhibition of the Acoustic Startle Response

Four sound-attenuated boxes (SR-Lab Startle Response System, San Diego Inst., San Diego, USA) were used. Each box consists of a Plexiglas cylinder situated on the top of a platform with a sensor that detects the strength made by the rat in each trial. Two speakers situated 15 cm from each side of the cylinder deliver the acoustic stimuli and a white noise generator provides the background noise. Each box is constantly lit by a 10 W lamp. The data are transduced by an accelerometer into a voltage which is amplified, digitized, and saved into a computer for further analysis.

The startle session starts with a 5 min habituation period in the startle chambers. Then, 10 "pulse-alone" trials (105 dB, 40 ms) are delivered in order to obtain a basal measure of the ASR (BASELINE 1). After this, each one of the six different types of trials are randomly administered 10 times (60 trials in total):

(1) Pulse-alone trials (105 dB 40 ms, BASELINE 2, this was the variable used to calculate the %PPI; see the formula below).
(2) Prepulses of 65/70/75/80 dB (20 ms) followed by the startle stimulus (105 dB, 40 ms), with an inter-stimulus interval of 100 ms.
(3) No stimulus trials (background noise 55 dB).

At the end, in order to measure the habituation to the startle stimulus, five "pulse-alone" trials were delivered (BASELINE 3).

The interval between trials was 10–20 s with a mean of 15 s. The startle magnitude was recorded during 200 ms after the onset of the pulse.

The degree of PPI (in percentage) is calculated according to the formula:

$$\%PPI = 100 - \left(\frac{startle\ amplitude\ on\ prepulse\ trials}{startle\ amplitude\ on\ pulse\ trials} \times 100 \right)$$

Spatial Working Memory (Delayed Matching-to-place Task; DMP) and Cued Learning in the Morris Water Maze

The Morris water maze test was performed in a circular water tank (140 cm in diameter and 30 cm deep) filled with water (24°C) made opaque with white paint.

The animals were tested on 3 consecutive days. They were allowed to swim for 90 s or until they located a platform (diameter 16 cm; height 28 cm) submerged (2 cm) in a fixed position each day. Each rat went through 2 trials per day: a sample/acquisition trial and a retention trial. The two trials were separated by 30 s and the rat was allowed to stay on the platform for 15 s and then spent another 15 s in an individual cage before the second trial started.

Three platform positions were defined: the first day the platform was located in the center of the NW quadrant, the second day it was located at a distance of 15 cm in the S direction and the third day the platform was at the center of the tank. Three starting positions were also defined: S, E, and W, respectively. The starting point and the location of the platform were pseudo-randomly varied each day.

Several room cues were constantly visible from the pool. Escape latencies, path lengths, and swimming speed from each rat and trial were provided by a tracking system (Smart v.2.5.14; PANLAB, Barcelona, Spain) connected to a video camera placed above the pool. Two variables (highly associated to each other) were computed as indexes of spatial working memory: "Mean T1-T2," distance savings in T2 vs. T1 (i.e., subtraction T1-T2) averaged for the 3 days. "Mean %DP T1-T2," difference of percentage of distance traveled in the periphery between T1 and T2, averaged for the 3 days.

Cued Learning

This task consisted of four consecutive trials at 15 min intervals on 2 consecutive days (i.e., 8 trials in total). For this test, the platform protruded 1 cm above the surface of the water and was cued with a small striped (black and white) flag. Black curtains were drawn to minimize the availability of extra-maze cues. There was one platform position (center of the SW quadrant) for the 2 days and 4 starting positions (N, S, E, W). The trials began with the rat facing the wall at the starting point and, if after the maximum allocated time (90 s) the animal had not found the platform, it was gently guided to its position by the experimenter. The parameter used in this task was the distance to reach the platform in each trial. This task is used to see if the animals had any visual, motor, or motivational problems.

Statistical Analysis

Statistical analysis was performed using the "Statistical Package for Social Science" (SPSS, version 17).

Pearson's correlation coefficients were performed among the main variables of both experiments. Multiple linear regression and factorial (direct oblimin; oblique rotation) analyses were applied to data from Experiment 2.

Repeated measures ANOVAs, with the 4 prepulse intensities as a within-subject factor ("3 strains" × "4 prepulse intensities" ANOVA) or with the 3 baseline startle trial blocks as within-subject factor ("3 strains" × "3 baseline startle blocks" ANOVA), were used to evaluate the results from the PPI session.

As differences in navigation speed were observed among the experimental groups (data not shown) in the working memory and cued learning tasks, we have taken the "distance traveled" to reach the platform as the main dependent variable from both experiments. For spatial working memory measures, repeated measures ANOVAs with "T1-T2" (mean distance traveled in the 3 first trials –T1- and three second trials –T2- of each trial pair) as within-subject factor ("3 groups" × "2 trial means" ANOVA) were applied. One-Way ANOVAs were then separately applied to T1 and T2 results (Experiment 1).

Analysis of "distance traveled through the periphery" along the 6 training trials of the working memory task and analysis of performance along the 8 trials of the cued learning task were carried out with the appropriate repeated measures ANOVAs with 6 or 8 trials as within-subject factors (i.e., "3 groups" × "6 trials" or "3 groups" × "8 trials"). *Post-hoc* LSD tests following significant ANOVA effects were applied for comparisons between groups.

Results

Experiment 1

Pearson's correlation coefficients between the main variables and for the three groups pooled are shown in **Table 1**. As expected, there are high within-test (or within-phase) correlations, ranging from $r = 0.73$ to $r = 0.83$ for baseline startle (and habituation) variables, and ranging from $r = 0.72$ to $r = 0.94$ for PPI parameters. There are also moderate correlations between the distance traveled in "T1" and "T2" and in the cued learning task (ranging from $r = 0.40$ to $r = 0.45$), but very low or no correlation between performance in the cued task and the working memory ("Mean T1-T2") index ($r = -0.02$ and $r = -0.29$). With regard to between-test associations, there are only moderate correlations among %PPI variables and "T2" performance in the working memory task (from $r = -0.31$ to $r = -0.38$).

The %PPI for each experimental group and prepulse intensity is represented in **Figure 1A**. The repeated measures ANOVA revealed a significant "strain" effect [$F_{(2, 62)} = 5.43$ $p < 0.007$; **Figure 1A**]. There was also the expected "prepulse intensity" effect [Huynh-Feldt $F_{(2.56, 166.54)} = 92.1$, $p < 0.001$], as %PPI increased with prepulse intensity (**Figure 1A**). One-Way ANOVA of the "Mean %PPI" for the 4 prepulse intensities revealed a significant "strain" effect [$F_{(2, 62)} = 5.43$ $p = 0.007$], and the LSD *post-hoc* tests showed that the RHA-I rats display lower mean %PPI than the other two strains (see **Figure 1B**).

A significant "strain" effect was also observed for baseline startle measures [$F_{(2, 62)} = 3.35$ $p = 0.041$; **Figure 1C**], which is apparently due to the fact that RLA-I rats display increased baseline startle along the three trial blocks in which the session was divided (in agreement with previous studies; López-Aumatell et al., 2009a,c). There was also a "trial block" effect [Huynh-Feldt $F_{(1.44, 93.5)} = 16.27$ $p < 0.001$], reflecting the habituation of the startle response along the session in the three experimental groups (**Figure 1C**].

In **Figure 2A** the average distance traveled in the first trials (T1) and in the second trials (T2) is represented. The repeated measures ANOVA analysis showed a "trial" effect [$F_{(1, 62)} = 13.9$, $p < 0.001$] and a significant "strain" effect [$F_{(2, 62)} = 5.74$ $p = 0.005$], mainly because RHA-I were overall worse than the other two groups (**Figure 2A**). Moreover, in order to control for the possible influence of "Mean DIST T1" we conducted a one-way analysis of covariance (ANCOVA), taking the mean distance saved between the first and second trials (Mean T1-T2) as the dependent variable and the "distance traveled in the first trials" (Mean DIST T1) as a covariate. This analysis yielded a significant "strain" effect [$F_{(2, 61)} = 6.39$ $p = 0.003$; **Figure 2B**], which is apparently due to a relative impairment of RHA-I rats in that measure.

We conducted repeated measures ANOVA on swimming speed data for each trial. A significant "strain" effect [$F_{(2, 62)} = 13.95$ $p < 0.001$] was observed, due to the fact that NIH-HS rats

TABLE 1 | Pearson's correlations among the main variables.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1-Baseline 1	1																
2-Baseline 2	**0.76****	1															
3-Baseline 3	**0.73****	**0.83****	1														
4-Habituation	**0.82****	**0.43****	**0.30***	1													
5-%PPI65	**0.26***	0.24	0.18	0.22	1												
6%PPI 70	**0.32****	**0.36****	**0.27***	0.23	**0.72****	1											
7-%PPI75	**0.25***	**0.27***	0.22	0.19	**0.87****	**0.79****	1										
8-%PPI80	0.18	0.19	0.11	0.16	**0.75****	**0.79****	**0.81****	1									
9-PPI Mean	0.29	**0.30***	0.23	0.23	**0.92****	**0.90****	**0.94****	**0.89****	1								
10-Mean DIST T1	−0.08	−0.05	−0.15	0.02	−0.18	−0.15	−0.11	−0.05	−0.15	1							
11-Mean DIST T2	−0.10	−0.08	−0.15	−0.03	**−0.38****	**−0.31***	**−0.35****	**−0.33****	**−0.38****	0.23	1						
12-Mean T1-T2	0.01	0.02	−0.02	0.03	0.13	0.10	0.16	0.20	0.15	**0.69****	**−0.55****	1					
13-Mean %DP T1	0.01	0.07	−0.09	0.05	−0.22	−0.10	−0.12	−0.12	−0.16	**0.64****	**0.38****	**0.27***	1				
14-Mean %DP T2	−0.00	0.05	0.00	−0.02	**−0.30***	−0.19	−0.23	**−0.29***	**−0.27***	**0.31***	**0.61***	−0.20	**0.59****	1			
15-Mean %DP T1-T2	0.01	0.02	−0.10	0.07	0.11	0.12	0.13	0.20	0.15	**0.32****	**−0.30***	**0.50****	**0.39****	**−0.52****	1		
(#)16-Cue day 1	−0.11	0.06	0.05	−0.08	**−0.42****	**−0.32***	**−0.29***	**−0.44****	**−0.41****	**0.40****	**0.44****	−0.02	**0.54****	**0.63****	−0.17	1	
(#)17-Cue day 2	0.06	**0.33***	−0.02	0.16	−0.22	−0.05	−0.12	−0.12	−0.15	0.09	**0.45****	**−0.29***	**0.45****	**0.53****	−0.15	**0.52****	1

*"Baseline 1," corresponds to the 10 first pulse-alone trials. "Baseline 2," corresponds to the 10 pulse-alone trials that are presented pseudorandomly in combination with the prepulse-pulse trials. "Baseline 3," refers to the last 5 pulse-alone trials. "Habituation" refers to the difference between the mean of the 5 pulse-alone trials presented in the beginning of the session and the mean of the last 5 pulse-alone trials. "%PPI65-%PPI80," percentage of response inhibition in the prepulse-pulse trials for each prepulse intensity. "PPI Mean," the global percentage inhibition averaged for the 4 prepulse intensities. "Mean DIST T1" and "Mean DistT2," mean distance traveled in the three first and the three second trials, respectively. "Mean T1-T2," distance savings in T2 vs. T1 (i.e., subtraction T1-T2) averaged for the 3 days. "Mean %DP T1" and "Mean %DP T2," mean of the percentage of the distance traveled in the periphery in the three first (T1) and second (T2) trials, respectively. "Mean %DP T1-T2," difference of percentage of distance traveled in the periphery between T1 and T2, averaged for the 3 days. "Cue day 1," mean distance traveled in the 4 trials of cued learning in the first day. "Cue day 2," mean distance traveled in the 4 trials of cued learning in the second day. Bold letter means significant Pearson's coefficient *$p < 0.05$ and **$p < 0.01$. $n = 65$. (#) $n = 41$.*

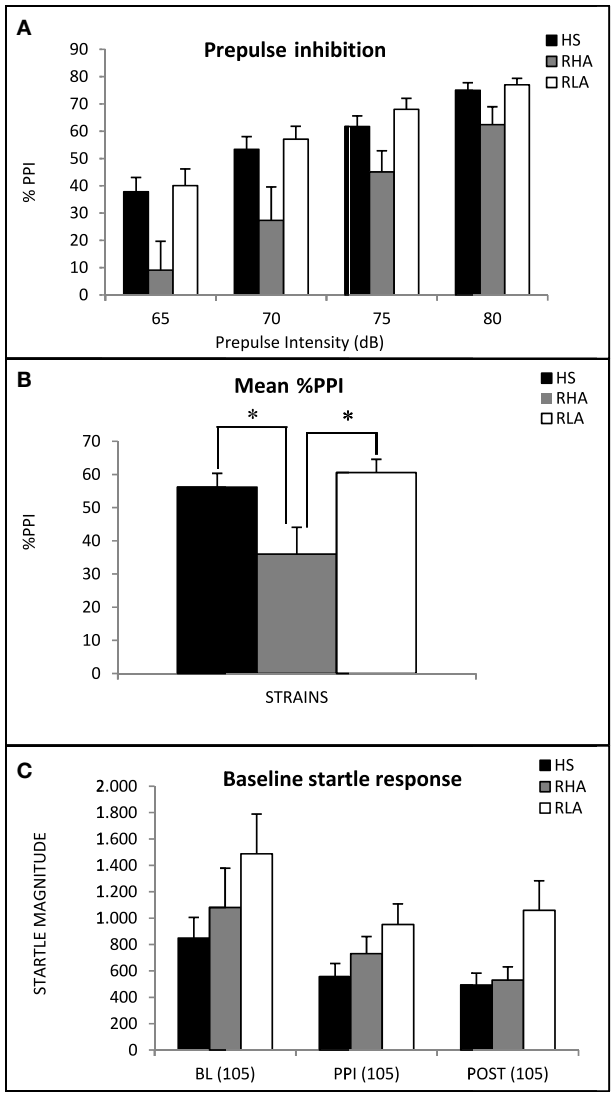


FIGURE 1 | (A) Mean prepulse inhibition (± S.E.M.) is shown for the three strains. **(B)** Mean ± S.E.M. of the "Mean %PPI" averaged for the four prepulse intensities. **(C)** Mean ± S.E.M. of the startle response in the three blocks of pulse-alone trials: BL(105), initial pulse-alone 10-trial block; PPI(105), second pulse-alone 10-trial block, pseudorandomly administered in combination with presentation of the prepulse-pulse trials; POST(105), the final pulse-alone 5-trial block. *$p < 0.05$ between the indicated groups (LSD tests).

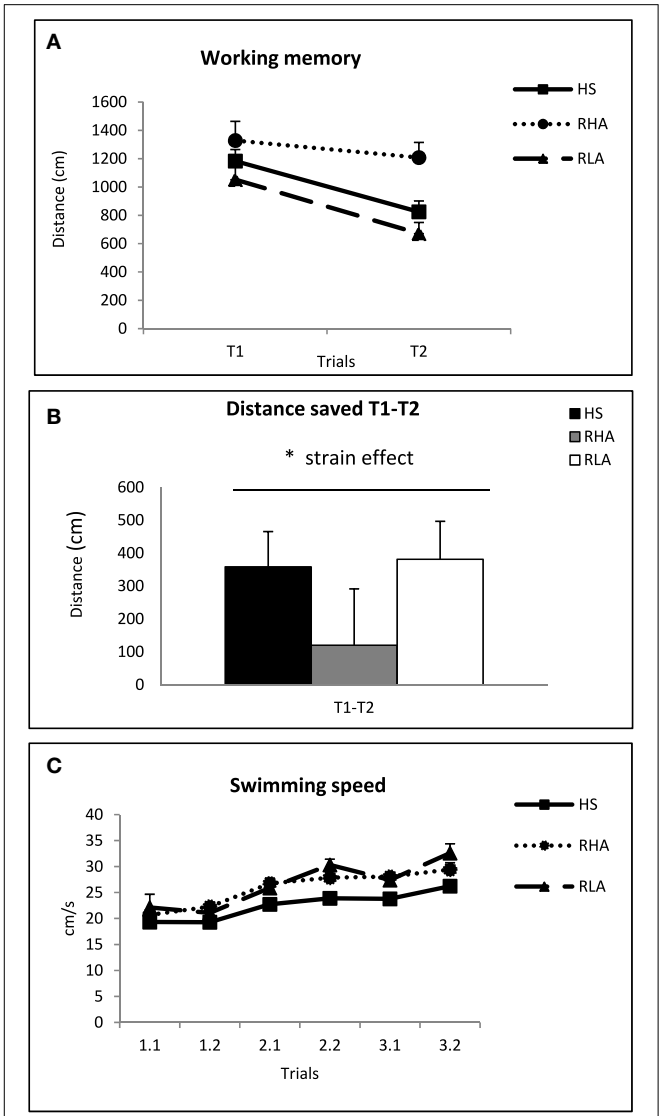


FIGURE 2 | (A) Mean ± S.E.M. of the distance (cm) traveled by the rats of the three strains in the first (T1) and second (T2) trials, averaged for the 3 days. **(B)** Mean ± S.E.M. of the three differences (i.e., subtractions T1-T2), corresponding to the 3 days, between the first (T1) and the second trials (T2) of the working memory task. **(C)** Mean ± S.E.M. swimming speed for each trial and strain. *$p < 0.05$, "Strain" effect (One-Way ANCOVA; see text).

swam apparently slower than both RHA-I and RLA-I groups (see **Figure 2C**).

The percentage of distance swam in the periphery, in each trial is shown in **Figure 3A**. In the repeated measures ANOVA we found a significant "strain" effect [$F_{(2, 62)} = 12.91$ $p < 0.001$]. "Trial" and "trial × strain" effects were also found [$F_{(5, 325)} = 15.17$, $p < 0.001$ and $F_{(10, 325)} = 5.04$, $p < 0.001$, respectively]. Further separate One-Way ANOVAs showed group effects in all trials except one [second trial in the first day, 1.2; $F_{(2, 62)} \geq 4.11$ $p \leq 0.021$ for the remaining five trials; **Figure 3A**]. *Post-hoc* LSD tests indicated that RHA-I rats swam longer distances in the periphery than the other two groups (trials 2.1, 2.2, and 3.2; **Figure 3A**) or than RLA-I rats (trial 3.1; see other LSD differences in **Figure 3A**). In accordance with the results observed for "Mean T1-T2" (**Figures 2A–C**), an ANOVA on the "difference of percentage of distance traveled in the periphery between T1 and T2 (averaged for the 3 days)" ("Mean %DP T1-T2") yielded a significant "strain" effect [$F_{(2, 62)} = 3.95$ $p < 0.024$; **Figure 3B**], and the LSD *post-hoc* tests revealed that RHA-I rats showed significantly lower values in that variable than NIH-HS rats (**Figure 3B**). Given that the "Mean %DP T1-T2" variable is highly and positively correlated/associated with "Mean T1-T2" (in both Experiments 1 and 2; see correlations between both variables in **Table 1** -$r = 0.50$-, **Table 4** -$r = 0.69$-, and the

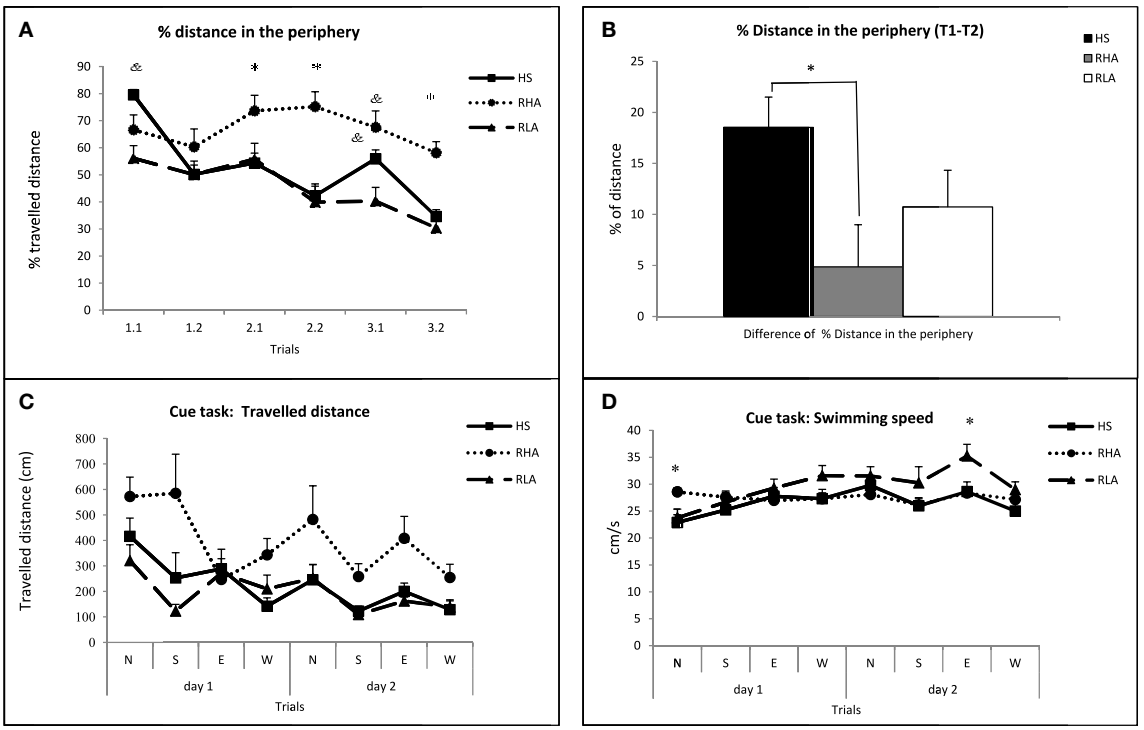


FIGURE 3 | (A) Mean ± S.E.M. of the percentage of distance traveled in the periphery in each trial for the three groups. **(B)** Mean ± S.E.M. of the difference of "percentage of distance traveled in the periphery between T1 and T2 (averaged for the 3 days)" ("Mean %DP T1-T2"). **(C)** Mean ± S.E.M. of the distance traveled by the rats in each of the 8 trials of the cued task. **(D)** Mean ± S.E.M. swimming speed in the cued task for each trial. *$p < 0.05$ vs. the other two strains **(A,D)** or between the groups indicated **(B)**; &, $p < 0.05$ vs. the RLA-I group (LSD tests following the corresponding significant ANOVA effects).

loadings of both variables −0.79 and 0.81- in the factor analysis from **Table 6B**), the impairment of RHA-I rats in the former variable suggest a relative deficit of RHA-I rats in a working memory-related process.

The repeated measures ANOVA of the distance traveled in the cued task (**Figure 3C**) revealed significant "strain" [$F_{(2,\ 38)} = 11.18$ $p < 0.001$] and "trial" [Huynh-Feldt $F_{(4.62,\ 175.38)} = 6.44$ $p < 0.001$] effects, apparently due to the longer distances traveled by RHA-I rats in some trials. Analysis of the swimming speed of the rats during the cued task (repeated measures ANOVA) indicated a significant "trial × strain" interaction [$F_{(14,\ 252)} = 2.01$ $p = 0.018$]. Further LSD *post-hoc* tests revealed significant differences between RHA-I rats and the other two strains in the first trial and between the RLA-I and the other two strains in the seventh trial (see LSD tests in **Figure 3D**).

Experiment 2

Table 2 shows the mean, S.E.M., and Standard Deviation (S.D.) %PPI of the 78 NIH-HS rats tested in the PPI session.

Table 3 shows the mean ± S.E.M of the three sub-groups of NIH-HS rats stratified on the basis of their mean %PPI performance with the 4 prepulse intensities. The 3 sub-groups were formed as follows: HIGH-PPI group ($n = 9$), consisting of rats showing total PPI scores 1 SD above the mean of the whole group ($n = 78$), i.e., PPI scores > 78%; LOW-PPI group ($n = 14$), consisting of rats showing total PPI scores 1 SD below the mean of the whole group, i.e., PPI scores < 42%; MEDIUM-PPI group ($n = 10$), consisting of randomly selected rats with total PPI scores falling within the mean ± 1 SD (see descriptives of the 3 subgroups in **Table 3**). The results listed in **Table 3** show that the 2 groups with extreme values of %PPI also diverge in the magnitude of the baseline startle response.

The correlation matrix in **Table 4** shows a pattern of high within-test correlations which is similar to that observed in **Table 1** (Experiment 1). Apart from these expected correlations it is remarkable that the "Mean T1-T2" is positively and significantly correlated with the PPI variables (i.e., %PPI70, %PPI75, %PPI80, and PPI Mean), with correlations ranging from 0.39 to 0.53. So, PPI is correlated with a typical measure of working memory such as the "Mean T1–T2." Another related correlation, also supporting the main hypothesis of the present study, is the one between the mean distance traveled in the second trial (Mean DIST T2) and the performance in the PPI when the prepulse had an intensity of 75 dB (−0.36). In the case of the other prepulse intensities the correlation with "Mean DIST T2" is not significant but there is a tendency, with Pearson's coefficients ranging from −0.25 to −0.33. Remarkably, the "difference of percentage of distance traveled in the periphery between T1 and T2 averaged for the 3 days" ("Mean % DP T1-T2"), which is highly correlated with the working memory

TABLE 2 | Mean, S.E.M. and Standard Deviation (S.D.) of the 78 NIH-HS rats tested in the PPI.

	Mean	*S.E.M*	*S.D.*
STARTLE RESPONSE			
Baseline 1	899.93	114.92	1014.96
Baseline 2	590.66	74.61	658.98
Baseline 3	474.84	58.74	518.76
Habituation	350.74	72.19	637.54
PPI			
%PPI65	41.29	3.20	28.24
%PP70	56.34	2.26	19.95
%PPI75	67.71	2.03	17.92
%PPI80	78.50	1.41	12.43
PPI Mean	60.96	2.01	17.78

*Symbols/abbreviations as in **Table 1**.*

TABLE 3 | Mean ± S.E.M. of the PPI and startle response variables of the 3 NIH-HS subgroups.

	Medium-PPI (n: 10)	High-PPI (n: 9)	Low-PPI (n: 14)
Baseline 1	606.24 ± 263.68	1550.98 ± 392.36*	571.63 ± 122.74
Baseline 2	256.84 ± 64.03	1194.12 ± 225.94*	361.22 ± 88.58
Baseline 3	348.54 ± 117.13	825.42 ± 254.31	381.64 ± 91.00
Habituation	352.66 ± 250.32	807.18 ± 349.05	303.04 ± 112.32
%PPI65	42.88 ± 4.74^a	74.98 ± 2.97^a	10.30 ± 4.26^a
%PP70	62.66 ± 4.39^a	83.77 ± 1.11^a	27.14 ± 3.88^a
%PPI75	68.84 ± 3.69^a	87.79 ± 0.91^a	39.91 ± 2.91^a
%PPI80	79.62 ± 3.82^a	$91.85 \pm 1,00^a$	60.88 ± 2.50^a
PPI Mean	63.50 ± 3.42^a	84.60 ± 1.11^a	34.56 ± 1.48^a

*Symbols/abbreviations as in **Table 1**. *$P < 0.05$ vs. the other 2 groups; $^a p < 0.05$ between groups with the same letter (LSD tests following significant One-Way ANOVAs; all [$Fs_{(2, 30)} \geq 4.57$ and $p \leq 0.019$].*

measure "Mean T1-T2" ($r = 0.69$), is also positively correlated with %PPI (i.e., %PPI75, %PPI80 and PPI Mean), with Pearson's coefficients ranging from 0.41 to 0.57. Moreover, correlations between measures of (total) distance (i.e., Mean DIST T1, Mean DIST T2) and "distance traveled in the periphery" (Mean %DP T1, Mean %DP T2) are significant, with coefficients ranging from 0.61 (between Mean DIST T2 and Mean %DP T2) to 0.68 (between Mean DIST T1 and Mean % DP T2; **Table 4**).

Forward stepwise multiple regression, with PPI variables as predictors and "Mean T1-T2" as the dependent variable revealed 2 significant models, with the first model showing that the variable %PPI75 is a significant predictor of "Mean T1-T2" (see **Figure 4A**, **Table 5**). %PPI75 correlated significantly with "Mean T1-T2," explaining 28% of its variability ($p = 0.002$; **Table 5**). In addition, a second significant regression model, with %PPI75 and %PPI65 as predictors (correlation of $r = 0.66$ with "Mean T1-T2") accounted for 44% of the variability ($p < 0.001$; **Table 5**). We also conducted the multiple regression analysis for the "Mean %DP T1-T2" and we observed that %PPI80 predicted "Mean %DP T1-T2" (**Figure 4B**; **Table 5**).

Obliquely-rotated factor analyses (direct oblimin) were performed with the 14 main variables of the two tests/tasks (**Table 6(A)**). The first result we obtained was a 4-factor structure (**Table 6(A)**). In the first factor we can see an association between PPI and working memory, with loadings of PPI variables ranging from 0.89 to 0.95 and with 0.43 and 0.41 loadings for "Mean T1-T2" and "Mean %DP T1-T2," respectively. The second factor is based on the startle response magnitude, particularly baseline startle, with loadings from 0.78 to 0.99. The third and fourth factors are related to the DMP task (loadings ranging from 0.41 to 0.91), with the main difference between the two factors being that in the third the distances in the first trials (Mean DIST T1 and Mean %DP T1) have the highest loadings, while in the fourth factor the distances in the second trials (Mean DIST T2 and Mean %DP T2) are the most relevant. Another important difference between these two factors is that "Mean T1-T2" has a higher loading in the third factor (0.74) while "Mean %DP T1-T2" has a -0.82 loading in the fourth factor. So, in this analysis we found that, with the exception of the first factor, the other three are predominantly task-related (or phase-related) factors (**Table 6(A)**).

In order to obtain a reduced number of theoretically meaningful factors the same factor analysis was reduced to a 2-factor solution (see **Table 6(B)**). In the first factor the PPI variables and the DMP task variables have the highest loadings, with 0.60–0.83 for %PPI65–%PPI80 and, remarkably, with 0.79 for "Mean T1-T2" and 0.81 for "Mean %DP T1-T2" (**Table 6(B)**). The second factor is essentially composed by the baseline startle variables (0.73–0.91) plus moderate loadings of %PPI65–%PPI75 variables (0.48–0.59). Thus, this factor analysis clearly links PPI and the working memory task in the first factor, while the second factor is mainly related to baseline startle response.

In **Figure 5A** we show the average distance traveled in the first (T1) and second (T2) trials by the three groups of NIH-HS rats. The repeated measures ANOVA showed that there is a significant interaction between "trial type" (T1 or T2) and NIH-HS rat "sub-group" [$F_{(2, 30)} = 3.44$ $p = 0.045$]. This interaction is important because it shows that the "LOW-PPI" group had a very small saving between T1 and in T2, while "HIGH-PPI" and "MEDIUM-PPI" groups show a clear cut decrease in the distance traveled between the two trials (**Figure 5A**). Further One-Way ANOVAs for each trial type revealed no significant differences between sub-groups. In **Figure 5B** the differences between T1 and T2 are shown for the three groups. One-Way ANOVA revealed significant differences between the three groups [$F_{(2, 30)} = 3.44$ $p = 0.045$], and *post-hoc* LSD tests evidenced that these differences were between the "LOW-PPI" group and the other two groups ($p < 0.05$; **Figure 5B**), indicating that the "LOW-PPI" sub-group displays a poorer working memory performance. Analysis of the swimming speed with a repeated measures ANOVA revealed a significant "sub-group" effect [$F_{(2, 30)} = 7.77$ $p = 0.002$], which is due to the fact that the LOW-PPI group was apparently slower than the other two sub-groups in some trials (**Figure 5C**).

The repeated measures ANOVA for the percentage of distance traveled in the periphery by the three NIH-HS sub-groups revealed no significant group effect [$F_{(2, 30)} = 0.70$ $p = 0.50$] and

TABLE 4 | Pearson's correlations among the main variables, pooling the three NIH-HS subgroups (n = 33).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1-Baseline 1	1																
2-Baseline 2	**0.83****	1															
3-Baseline 3	**0.86****	**0.79****	1														
4-Habituation	**0.81****	**0.51****	**0.46****	1													
5-%PPI65	**0.45****	**0.53****	**0.37***	0.33	1												
6-%PPI 70	**0.35***	**0.44***	0.23	0.23	**0.86****	1											
7-%PPI75	0.29	**0.41***	0.23	0.16	**0.83****	**0.85****	1										
8-%PPI80	0.18	**0.37***	0.14	0.10	**0.73****	**0.78****	**0.85****	1									
9-PPI Mean	**0.36***	**0.48****	0.28	0.24	**0.94****	**0.95****	**0.94****	**0.88****	1								
10-Mean DIST T1	0.21	0.23	0.13	0.23	0.04	0.21	**0.36***	**0.37***	0.23	1							
11-Mean DIST T2	0.09	0.01	0.10	0.15	−0.25	−0.32	**−0.36***	−0.33	−0.33	0.08	1						
12-Mean T1-T2	0.10	0.17	0.03	0.07	0.21	**0.39***	**0.53****	**0.52****	**0.42***	**0.70****	**−0.65****	1					
13-Mean %DP T1	**0.36***	0.34	0.27	0.29	0.20	0.31	**0.37***	**0.38***	0.32	**0.68****	0.14	**0.42***	1				
14-Mean %DP T2	0.09	0.02	0.05	0.13	−0.09	−0.08	−0.15	−0.23	−0.13	0.18	**0.61****	−0.30	**0.50****	1			
15-Mean %DP T1-T2	0.20	0.26	0.18	0.01	0.26	0.34	**0.48****	**0.57****	**0.41***	**0.38***	**−0.56****	**0.69****	0.28	**−0.69****	1		
(#) 16-Cue Day 1	0.36	0.21	0.32	0.37	0.17	0.13	0.22	−0.01	0.15	0.32	0.18	0.13	**0.66****	**0.66****	−0.27	1	
(#) 17-Cue Day 2	−0.01	0.32	−0.15	0.01	**0.47***	0.41	0.41	0.29	0.44	−0.12	0.18	−0.20	0.15	0.19	−0.11	0.13	1

*Symbols/abbreviations as in **Table 1**. Bold letter means significant Pearson's coefficient *$p < 0.05$ and **$p < 0.01$. (#) $n = 17$.*

a significant trial effect [$F_{(5,\ 150)} = 28.6\ p < 0.001$] (**Figure 6A**). On the other hand, One-Way ANOVA of the "Mean %DP T1-T2" showed a significant "sub-group" effect [$F_{(2,\ 30)} = 4.51\ p < 0.019$], and the LSD *post-hoc* tests revealed that the differences were between LOW-PPI and HIGH-PPI groups, with the former traveling longer distances in the periphery (**Figure 6B**).

In **Figure 6C** we show the distance traveled in the 8 trials of the cued task. The results of the repeated measures ANOVA showed a significant group effect [$F_{(2,\ 14)} = 4.37\ p = 0.033$] and a trial effect [Huynh-Feldt $F_{(3.62,\ 50.78)} = 2.88\ p = 0.036$; **Figure 6C**], which is apparently due to HIGH-PPI group traveling longer distances in some trials (see **Figure 6C**). Swimming speed along the 8 trials was analyzed with a repeated measures ANOVA, that only revealed a "trial" effect [$F_{(7,\ 98)} = 3.96\ p = 0.001$; **Figure 6D**].

Discussion

Experiment 1 represents the first joint characterization of PPI and spatial working memory in RHA-I/RLA-I and NIH-HS rats. In keeping with the hypothesis that RHA-I rats could be a tool to discern cognitive peculiarities linked to some schizophrenia-related symptoms, we have found that this rat strain displays deficits in PPI and in spatial working memory compared with RLA-I and NIH-HS rats. In addition, it is also shown for the first time that the genetically heterogeneous NIH-HS rat stock displays relatively high PPI levels as well as efficient spatial working memory, as their values in both processes/measures are similar to those shown by RLA-I rats (see Experiment 1). The results from Experiment 1 and Experiment 2 also point out an apparent positive association between PPI and working memory, as the strains (i.e., RLA-I, NIH-HS; Experiment 1) or sub-groups (i.e., the HIGH-PPI and MEDIUM-PPI sub-groups of NIH-HS rats; Experiment 2) displaying the highest PPI levels also present the best working memory performance.

RLA rats from the Swiss outbred line have already been shown to outperform their RHA counterparts in spatial learning/memory tasks (e.g., Driscoll et al., 1995; Escorihuela et al., 1995b; Aguilar et al., 2002). Accordingly, we recently found that inbred RHA-I rats performed worse than RLA-I animals in a spatial place task (i.e., reference memory) in the Morris Water maze (Martínez-Membrives et al., 2015). But the present is the first time in which (spatial) working memory has been assessed in the inbred Roman rat strains, showing that the RHA-I strain displays impaired memory ability in this task as compared with both RLA-I and NIH-HS rats. Remarkably, as we have reported for anxiety/stress-related behavioral/neuroendocrine traits (e.g., López-Aumatell et al., 2009a,b; Díaz-Morán et al., 2012, 2013c; Estanislau et al., 2013), PPI and working memory performance of the heterogeneous NIH-HS rat stock very closely resemble the profile of RLA-I rats in these tests/tasks. These similar profiles, in both a pre-attentive process (PPI) and spatial working memory, jointly with their comparable performance in cued learning, in spatial place learning and in two-way avoidance acquisition (e.g., López-Aumatell et al., 2009a,b; Díaz-Morán et al., 2012, 2013c; Estanislau et al., 2013; Martínez-Membrives et al., 2015), suggest that inbred RLA-I and heterogeneous NIH-HS rats may also present similarities in some other cognitive traits or profiles. Further studies aimed at comparing the performance of RLA-I/RHA-I and NIH-HS rats in other cognitive/executive tasks are therefore warranted.

The RHA-I rat strain also showed a poorer performance in the cue learning task, a test that is commonly used to rule out possible sensorial, motivational, or motor deficits. This result was unexpected, because we have recently found that RHA-I and RLA-I rats present identical performance in a cue

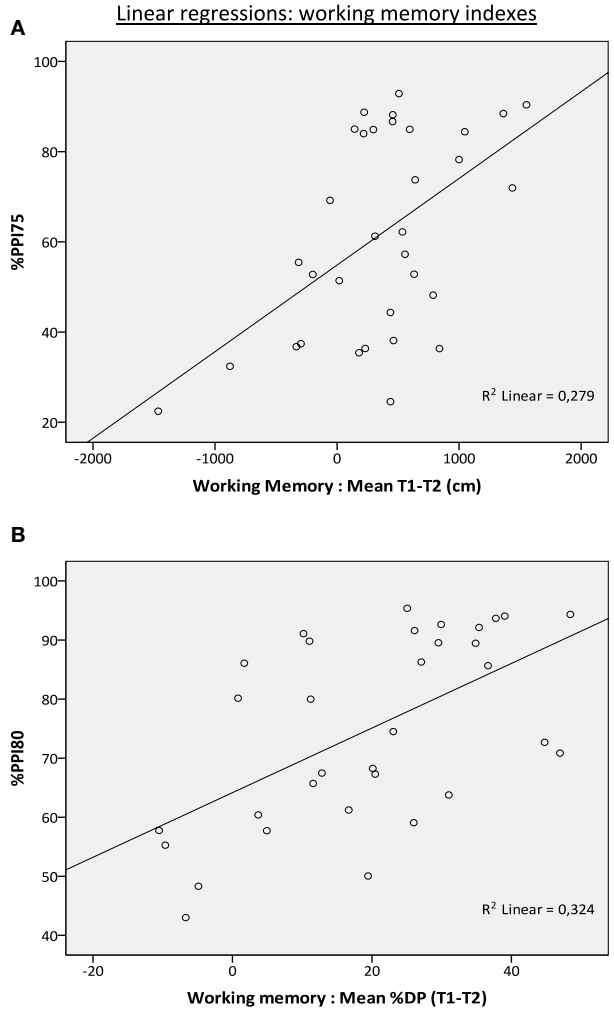


FIGURE 4 | Linear regressions between %PPI and "working memory" measures (all rats pooled, $n = 33$). (A) Regression between %PPI75 (predictor variable) and "Mean T1-T2" (dependent variable). **(B)** Regression between %PPI80 (predictor variable) and "Mean %DP T1-T2" (dependent variable).

task administered after a place task in the MWM (Martínez-Membrives et al., 2015). Apart from that recent work, several studies performed at our laboratory and others allow us to rule out the possibility of any visual, motivational, or motor deficit in RHA-I rats while, on the contrary, it is well-known that they are characterized by: (i) enhanced exploratory drive (motivation) and a novelty seeker profile, as observed in many different novelty situations (e.g., reviews by Fernández-Teruel et al., 1992; Escorihuela et al., 1999; Driscoll et al., 2009); (ii) augmented impulsivity and tendency to stereotyped behavior (Zeier et al., 1978; Moreno et al., 2010; Klein et al., 2014) and (iii) enhanced perseverative responses and/or lack of behavioral flexibility (e.g., Zeier et al., 1978; Nil and Bättig, 1981; Escorihuela et al., 1995b; Moreno et al., 2010). Finally, the "strain × trial" effect (and the absence of "strain" effects) on navigation speed in the cued task (Experiment 1) do not suggest any motivational deficit which could account for the performance results observed. Thus, although it is not the only possible interpretation (see below), the impairment of RHA-I rats in the cue task might be partly due to the fact that they perseverate in (spatial or/and non-spatial) swimming strategies acquired during the working memory task.

In that context, it is worth noting that in both the working memory and cued tasks RHA-I rats display increased circular navigation along the periphery of the pool. This may suggest enhanced thigmotaxis in that rat strain. Nevertheless, we know, from a number of studies using open field-like tests (e.g., open field, activity cages, hole-board), that RHA-I rats spend the same time close to the walls (i.e., in the periphery) and travel the same percentage of distance along the periphery as RLA-I rats (e.g., Estanislau et al., 2013, and unpublished results from our laboratory). Hence, thigmotactic-like behavior is neither an inherent nor a general trait in RHA-I rats but, still, they display an excessive amount of peripheral navigation in the present MWM tasks. Thus, there is the possibility that a working memory deficit in the RHA-I rats may not be the only interpretation of the present results, and that a non-optimal navigation/orientation strategy (which would be in agreement with RHA vs. RLA results in other aquatic spatial tasks; Nil and Bättig, 1981) could have a role in the increased total distance and "% distance in the periphery" traveled by the RHA-I strain.

The results of Experiment 1 also highlight that the RHA-I strain fulfills some criteria for being considered as a convenient model for studying some schizophrenia-relevant symptoms, since it displays clear PPI and (possibly) working memory deficits along with a relative deficit in latent inhibition (Fernández-Teruel et al., 2006), an impaired performance in the five-choice serial reaction time task (5-CSRTT; which reflects executive function and sustained attention; Moreno et al., 2010; Klein et al., 2014) and an increased sensitivity to acutely administered DA receptor agonists as well as to psychostimulant (DA agonist)-induced sensitization (e.g., Corda et al., 2005; Giménez-Llort et al., 2005; Giorgi et al., 2007; Guitart-Masip et al., 2008; Del Río et al., 2014).

Experiment 2 was devoted to further investigate such a PPI-working memory association by evaluating whether PPI levels would statistically predict memory performance in a sample of the most genetically heterogeneous laboratory rat in existence, i.e., the NIH-HS rats (e.g., Baud et al., 2013, 2014a,b; Johannesson et al., 2009). Thus, the expected advantage of addressing that issue in NIH-HS rats would be that their enhanced genetic variability could make results more generalizable than those obtained using typical laboratory rodent strains. The results of Experiment 2 clearly support a positive relationship between PPI and working memory, as shown by the memory performance of HIGH-PPI as compared with MEDIUM-PPI and LOW-PPI rats, as well as by the positive associations between PPI (at different prepulse intensities) and the working memory measures revealed by correlational, factorial, and regression analyses (see **Tables 4–6** and **Figure 4**). In fact, Pearson's correlations between working memory ("Mean T1-T2" index) and %PPI70, %PPI75, %PPI80, and mean %PPI range from $r = 0.39$ to $r = 0.53$ (**Table 4**). Likewise, the stepwise multiple regression analysis

TABLE 5 | Forward stepwise multiple regression performed with "Mean T1-T2" and "Mean %DP T1-T2" as the dependent variables, and including all the variables recorded in the PPI session (i.e., Baseline 1–3, Habituation and %PPI65–%PPI80) as predictors in the model.

Dependent variable	Method	Model	Predictor variables	*R*	R^2	*p*
Mean T1-T2	Forward Stepwise	1	%PPI75	0.53	0.28	0.002
Mean T1-T2	Forward Stepwise	2	%PPI75; %PPI65	0.66	0.44	< 0.001
Mean %DP T1-T2	Forward stepwise	1	%PPI80	0.57	0.32	0.001

Mean T1-T2 is predicted by the PPI variables %PPI75 and %PPI65. Mean %DP T1-T2 is predicted by %PPI80. The three NIH-HS subgroups (n = 33) are pooled for analysis.

TABLE 6 | Loadings ≥ 0.40 are shown.

(A)	Factor 1	Factor 2	Factor 3	Factor 4	(B)	Factor 1	Factor 2
Baseline 1	–	0.99	–	–	Baseline 1	–	0.91
Baseline 2	0.47	0.87	–	–	Baseline 2	–	0.84
Baseline 3	–	0.89	–	–	Baseline 3	–	0.78
Habituation	–	0.78	–	–	Habituation	–	0.73
%PPI65	0.92	0.45	–	–	%PPI65	0.60	0.59
%PPI70	0.95	–	–	–	%PPI70	0.71	0.52
%PPI75	0.94	–	–	–	%PPI75	0.81	0.48
%PPI80	0.89	–	0.41	–	%PPI80	0.83	–
Mean DIST T1	–	–	0.94	–	Mean DIST T1	–	0.41
Mean DIST T2	–	–	–	0.84	Mean DIST T2	−0.72	–
Mean T1-T2	0.43	–	0.74	−0.64	Mean T1-T2	0.79	–
Mean %DP T1	–	–	0.85	–	Mean %DP T1	–	0.60
Mean %DP T2	–	–	–	0.91	Mean %DP T2	−0.55	–
Mean %DP T1-T2	0.41	–	0.44	−0.82	Mean %DP T1-T2	0.81	–
% of variance (cumulative)	39.40	61.01	74.52	85.50	% of variance (cumulative)	39.40	61.01
Correlations	1				Correlation	0.17	
	0.31	1			N =	33	
	0.27	0.18	1				
	−0.27	0.07	−0.07	1			
N =	33						

(A) Oblique four-factor solution (direct oblimin) with the main behavioral variables and correlations between factors. Only factors with eigenvalues greater than 1 are considered. (B) Two-factor solution and correlation between factors, showing that both factors are almost orthogonal/independent. Symbols/abbreviations as in ***Table 1****. n = 33 (the three NIH-HS subgroups are pooled for the analyses).*

supports this correlational pattern, as the %PPI75 and %PPI65 are significant predictors of both working memory measures, "Mean T1-T2" and "Mean %DP T1-T2" (see **Table 5** and **Figures 4A,B**). Further supporting these results, the factor analysis (oblimin direct; unforced rotation) shows an initial four-fold solution in which, (1) the first factor combines high loadings of %PPI variables (0.89–0.95) with moderate loadings of the working memory variables (i.e., "Mean T1-T2," loading 0.43; "Mean %DP T1-T2," loading 0.41) (**Table 6(A)**); (2) a second factor related to the startle response variables and (3) the last two factors related to the distance variables measured in the MWM. Likewise, when forcing this factor analysis to a two-fold solution (direct oblimin), a very similar first factor is observed, with high loadings of %PPI variables and working memory indexes ("Mean T1-T2"and "Mean %DP T1-T2"), and also high loading of distance traveled in the second trials ("Mean DIST T2" and "Mean %DP T2"). The second and almost orthogonal factor (as the between-factor correlation is low) is mainly grouping loadings of baseline startle, habituation, and moderate loadings in the PPI variables and the distance traveled in the first trials in the MWM (**Table 6(B)**).

Thus, correlational, regression, and factor analyses confirm that: (1) the differences of % distance traveled in the periphery between T1 and T2 ("Mean %DP T1-T2") are strongly associated to "Mean T1-T2," so both of them are working memory indexes; and (2) both working memory variables are positively predicted by %PPI.

As concerns to animal research, to the best of our knowledge the PPI-working memory association has only been addressed in one study in mice and no study has evaluated it in untreated/undisturbed genetically heterogeneous rats. Singer et al. (2013) reported, using a cohort of 23 male C57BL/6 mice, that PPI levels positively predicted (with $r = 0.50$) spatial working memory in the Morris Water maze (delayed matching-to-place task). The authors showed that such an effect was only present when taking as predictor the %PPI levels at the lowest pre-pulse intensity (i.e., 69 dB). Our present

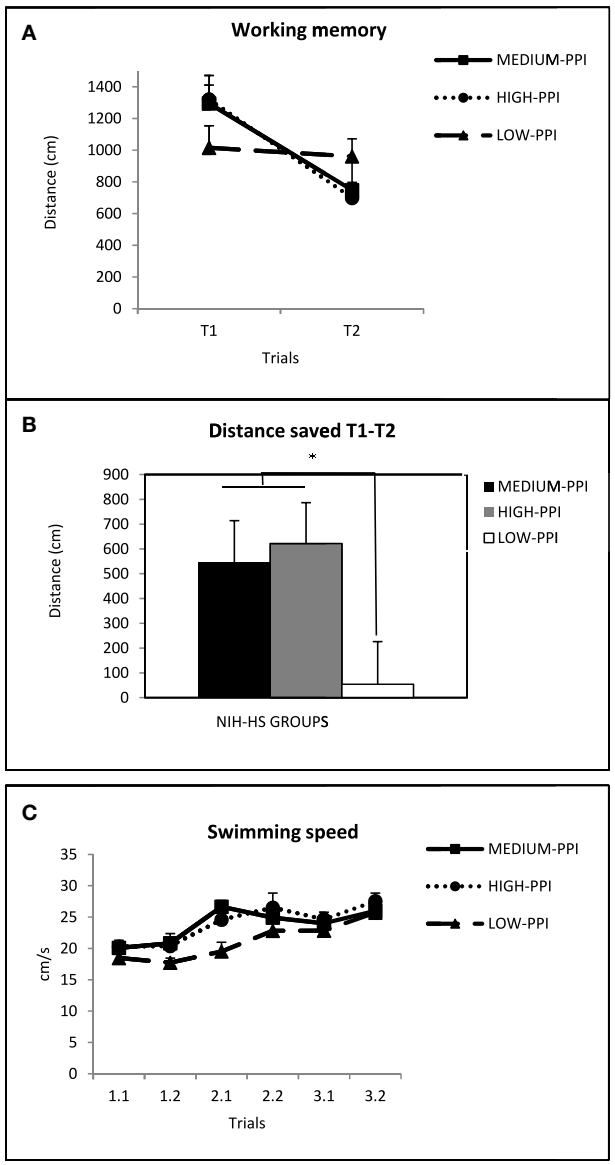


FIGURE 5 | (A) Mean ± S.E.M. of the distance (cm) traveled by the three subgroups of NIH-HS rats (selected for their extreme performance in the PPI test) in the first (T1) and second trials (T2), averaged for the 3 days (see text for the criteria followed to build these 3 subgroups). **(B)** Mean ± S.E.M. of the three differences (i.e., "Mean T1-T2"), corresponding to the 3 days, between the first (T1) and second (T2) trials of the working memory task. **(C)** Mean ± S.E.M. swimming speed of the NIH-HS sub-groups for each trial.*$p < 0.05$ between the indicated groups (LSD test following significant One-Way ANOVA).

results confirm and extend the previous study in mice to different rat types and statistical approaches, as we also found a positive association between %PPI and spatial working memory by comparing the RHA-I, RLA-I, and NIH-HS strains/stocks and by studying PPI-working memory associations in NIH-HS rats.

Remarkably, Experiment 2 shows that cue learning is neither associated with %PPI nor with working memory performance in NIH-HS rats (see **Table 4** and **Figure 6B**). Finally, the extreme LOW-PPI rats from such stock presented a pattern of results both across the different intensities of the prepulse inhibition study and on working memory measures at the DMP test, which paralleled those of the Roman High-Avoidance rats in Experiment 1. This suggests that either by psychogenetic selection or by focusing on extremes from a heterogeneous rats stock, it is possible to detect a useful (perhaps "at risk") phenotype to study cognitive peculiarities linked to some schizophrenia anomalies.

It has been proposed that PPI may be associated to, and modulated by, higher cognitive processes. This contention is supported by the frequent co-existence of PPI deficits and cognitive impairments in clinical samples, including schizophrenic patients, as well as by some studies in healthy volunteers which have shown that PPI is positively correlated with performance in several cognitive tasks (e.g., Bitsios and Giakoumaki, 2005; Hagan and Jones, 2005; Bitsios et al., 2006; Giakoumaki et al., 2006; Csomor et al., 2009; for reviews see Young et al., 2009; Singer et al., 2013). In particular, in human volunteers, PPI has been found to be positively associated to proper searching strategies in the CANTAB (*"Cambridge Neuropsychological Test Automated Battery")* spatial working memory task, Csomor et al., 2009). In spite of these positive results, the possibility that PPI predicts cognitive function in humans remains to be established (Young et al., 2009; Singer et al., 2013), given the small number of studies that have addressed that issue.

Koch and Schnitzler (1997) have proposed that the essential circuit underlying PPI involves the midbrain inferior colliculus, the superior colliculus, the pedunculopontine tegmental nucleus, and the caudal pontine reticular nucleus, which regulates the activity of motor neurons and the motor response (Koch and Schnitzler, 1997; Kohl et al., 2013). Importantly, however, cortical, and limbic areas, such as the orbitofrontal cortex, anterior cingulate, medial prefrontal cortex, nucleus accumbens, basolateral amygdala, and the hippocampus are known to modulate PPI or to affect its regulation in different ways, as reflected by disruption of PPI following manipulations of these structures (for review see Kohl et al., 2013). Spatial working memory, as assessed in the DMP task, is known to be hippocampus dependent (Whishaw, 1985; Morris et al., 1986a,b; Wible, 2013). Thus, the finding that PPI and spatial working memory can be modulated by hippocampal function, albeit to a different extent or in different ways, together with the important role attributed to the hippocampus in other schizophrenia-relevant cognitive processes and schizophrenic symptoms (e.g., Gray et al., 1991; Sawa and Snyder, 2002; Wible, 2013), suggests that the hippocampus represents a prime candidate structure to investigate neurobiological processes underlying particular symptom clusters. In this connection, it is remarkable that the RLA-I rat strain has a more functional hippocampus and higher hippocampal neuronal density than the RHA-I strain (Meyza et al., 2009; Garcia-Falgueras et al., 2012), which may underlie the better performance of RLA-I rats in both PPI and spatial working memory.

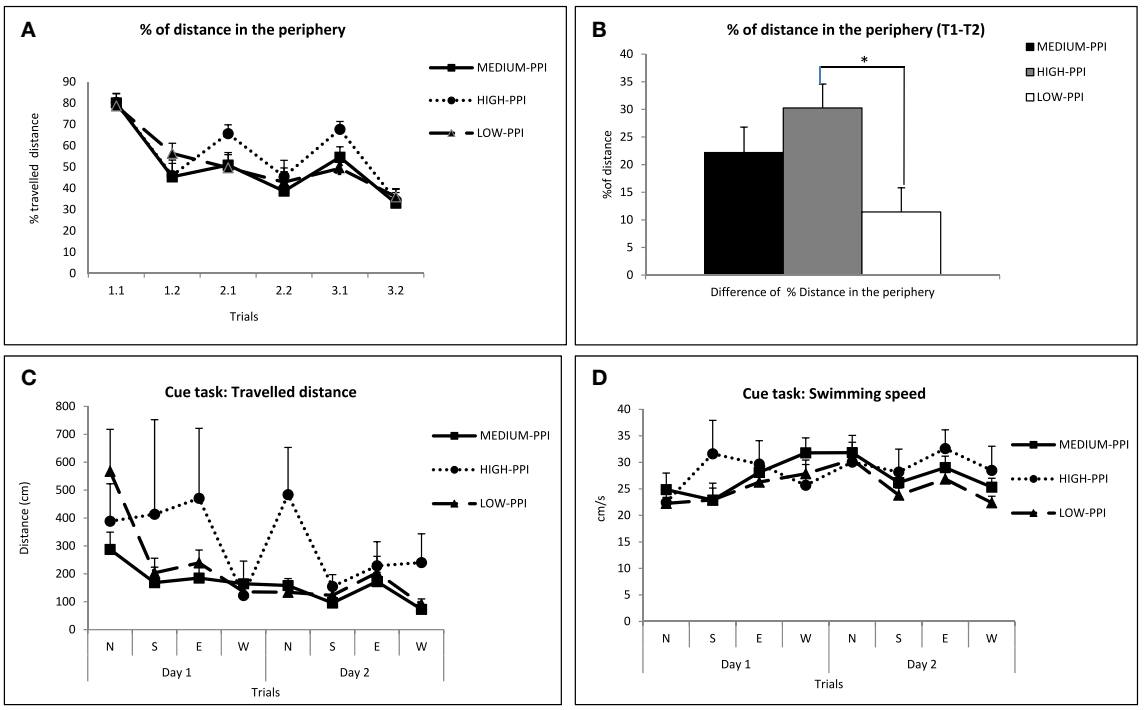


FIGURE 6 | (A) Mean ± S.E.M. of the percentage of distance traveled in the periphery in each trial for the 3 groups of NIH-HS rats. **(B)** Mean ± S.E.M. of the "difference of percentage of distance traveled in the periphery between T1 and T2 (averaged for the 3 days)" (i.e., "Mean %DP T1-T2"). **(C)** Mean ± S.E.M. of the distance (cm) traveled by the rats of the three groups in each of the 8 trials of the cued task. **(D)** Mean ± S.E.M. swimming speed of the NIH-HS sub-groups for each trial. *$p < 0.05$ between the groups indicated (LSD test following significant One-Way ANOVA).

In summary, the present study demonstrates a consistent and positive PPI-working memory association using three different strategies: (1) comparing three strains/stocks of rats which show differential PPI levels (Experiment 1); (2) evaluating working memory in subsamples of NIH-HS rats displaying extreme scores in PPI; and (3) performing correlational, regression, and factor analysis of PPI and working memory assessed in a sample of genetically heterogeneous NIH-HS rats. The results of the present study, together with those from Singer et al. (2013) in mice, support the idea that PPI and working memory are positively associated in untreated animals, thus paving the path for the study of possible common neurobiological mechanisms of pre-attentive (sensorimotor gating) and higher cognitive processes in rodents that can illuminate routes for abnormal functioning in schizophrenias.

Acknowledgments

This work was supported by grants PSI2013-41872-P (MINECO), 2014SGR-1587 (DGR), "ICREA-Academia 2013" (to AF), and partially supported by grants from the Italian Ministry of the University and Research (MIUR) to OG and MC and from ARS (Autonomous Region of Sardinia) (L.R.7/2007, "Promotion of Scientific Research and Technological Innovation in Sardinia," European Social Fund, 2007–2013, Project code no. CRP-59842) to OG. IO is recipient of a PhD FI fellowship (DGR 2014).

References

Aguilar, R., Escorihuela, R. M., Gil, L., Tobeña, A., and Fernández-Teruel, A. (2002). Differences between two psychogenetically selected lines of rats in a swimming pool matching-to-place task: long-term effects of infantile stimulation. *Behav. Genet.* 32, 127–134. doi: 10.1023/A:1015253807488

Alam, I., Koller, D. L., Cañete, T., Blázquez, G., López-Aumatell, R., Martínez-Membrives, E., et al. (2014). High-resolution genome screen for bone mineral density in heterogeneous stock rat. *J. Bone Miner Res.* 7, 1619–1626. doi: 10.1002/jbmr.2195

Baud, A., Flint, J., Fernández-Teruel, A., and The Rat Genome Sequencing Mapping and Consortium (2014b). Identification of genetic variants underlying anxiety and multiple sclerosis in heterogeneous stock rats. *World J. Neurosci.* 4, 216–224. doi: 10.4236/wjns.2014.43025

Baud, A., Hermsen, R., Guryev, V., Stridh, P., Graham, D., McBride, M. W., et al. (2013). Combined sequence-based and genetic mapping analysis of complex traits in outbred rats. *Nat. Genet.* 45, 767–775. doi: 10.1038/ng.2644

Baud, A., Guryev, V., Hummel, O., Johannesson, M., The Rat Genome Sequencing and Mapping Consortium, and Flint, J. (2014a). Genomes and phenomes of a population of outbred rats and its progenitors. *Sci. Data* 1:140011. doi: 10.1038/sdata.2014.11

Bentareha, R., Araujo, F., Ruano, D., Driscoll, P., Escorihuela, R. M., Tobeña, A., et al. (1998). Pharmacological properties of the GABAA receptor complex from brain regions of (hypoemotional) Roman high- and (hyperemotional)

low-avoidance rats. *Eur. J. Pharmacol.* 354, 91–97. doi: 10.1016/S0014-2999(98)00428-2

Bignami, G. (1965). Selection for high rates and low rates of avoidance conditioning in the rat. *Anim. Behav.* 13, 221–227. doi: 10.1016/0003-3472(65)90038-2

Bitsios, P., and Giakoumaki, S. G. (2005). Relationship of prepulse inhibition of the startle reflex to attentional and executive mechanisms in man. *Int. J. Psychophysiol.* 55, 229–241. doi: 10.1016/j.ijpsycho.2004.08.002

Bitsios, P., Giakoumaki, S. G., Theou, K., and Frangou, S. (2006). Increased prepulse inhibition of the acoustic startle response is associated with better strategy formation and execution times in healthy males. *Neuropsychologia* 44, 2494–2499. doi: 10.1016/j.neuropsychologia.2006.04.001

Broadhurst, P. L., and Bignami, G. (1965). Correlative effects of psychogenetic selection: a study of the Roman High and Low Avoidance strains of rats. *Behav. Res. Ther.* 3, 273–280.

Carrasco, J., Márquez, C., Nadal, R., Tobeña, A., Fernández-Teruel, A., and Armario, A. (2008). Characterization of central and peripheral components of the hypothalamus-pituitary-adrenal axis in the inbred Roman rat strains. *Psychoneuroendocrinology* 33, 437–445. doi: 10.1016/j.psyneuen.2008.01.001

Corda, M. G., Lecca, D., Piras, G., Di Chiara, G., and Giorgi, O. (1997). Biochemical parameters of dopaminergic and gabaergic neurotransmission in the CNS of Roman High-avoidance and Roman Low-avoidance rats. *Behav. Genet.* 27, 527–536. doi: 10.1023/A:1021452814574

Corda, M. G., Piras, G., Lecca, D., Fernández-Teruel, A., Driscoll, P., and Giorgi, O. (2005). The psychogenetically selected Roman rat lines differ in the susceptibility to develop amphetamine sensitization. *Behav. Brain Res.* 157, 147–156. doi: 10.1016/j.bbr.2004.06.016

Cromwell, H. C., Mears, R. P., Wan, L., and Boutros, N. N. (2008). Sensory gating: a translational effort from basic to clinical science. *Clin. EEG Neurosci.* 39, 69–72. doi: 10.1177/155005940803900209

Csomor, P. A., Yee, B. K., Feldon, J., Theodoridou, A., Studerus, E., and Vollenweider, F. X. (2009). Impaired prepulse inhibition and prepulse-elicited reactivity but intact reflex circuit excitability in unmedicated schizophrenia patients: a comparison with healthy subjects and medicated schizophrenia patients. *Schizophr. Bull.* 35, 244–255. doi: 10.1093/schbul/sbm146

Del Río, C., Oliveras, I., Cañete, T., Blázquez, G., Tobeña, A., and Fernández-Teruel, A. (2014). Genetic rat models of schizophrenia-relevant symptoms. *World J. Neuroscience.* 4, 261–278. doi: 10.4236/wjns.2014.43030

Díaz-Morán, S., Estanislau, C., Cañete, T., Blázquez, G., Ráez, A., Tobeña, A., and Fernández-Teruel, A. (2014). Relationships of open-field behaviour with anxiety in the elevated zero-maze test: focus on freezing and grooming. *World J. Neurosci.* 4, 1–11. doi: 10.4236/wjns.2014.410

Díaz-Morán, S., Martínez-Membrives, E., López-Aumatell, R., Cañete, T., Blázquez, G., Palencia, M., et al. (2013c). What can we learn on rodent fearfulness/anxiety from the genetically heterogeneous NIH-HS rat stock? *Open J. Psychiatry.* 3, 238–250. doi: 10.4236/ojpsych.2013.32022

Díaz-Morán, S., Palència, M., Mont-Cardona, C., Cañete, T., Blázquez, G., Martínez-Membrives, E., et al. (2012). Coping style and stress hormone responses in genetically heterogeneous rats: comparison with the Roman rat strains. *Behav. Brain Res.* 228, 203–210. doi: 10.1016/j.bbr.2011.12.002

Díaz-Morán, S., Palència, M., Mont-Cardona, C., Cañete, T., Blázquez, G., Martínez-Membrives, E., et al. (2013a). Gene expression in hippocampus as a function of differential trait anxiety levels in genetically heterogeneous NIH-HS rats. *Behav. Brain Res.* 257, 129–139. doi: 10.1016/j.bbr.2013.09.041

Díaz-Morán, S., Palència, M., Mont-Cardona, C., Cañete, T., Blázquez, G., Martínez-Membrives, E., et al. (2013b). Gene expression in amygdala as a function of differential trait anxiety levels in genetically heterogeneous NIH-HS rats. *Behav. Brain Res.* 252, 422–431. doi: 10.1016/j.bbr.2013.05.066

Driscoll, P., and Bättig, K. (1982). "Behavioural, Emotional and neurochemical profiles of rats selected for extreme differences in active, two-way avoidance performance," in *Genetics of the Brain*, ed I. Lieblich (Amsterdam: Elsevier), 95–123.

Driscoll, P., Fernández-Teruel, A., Corda, M. G., Giorgi, O., and Steimer, T. (2009). "Some guidelines for defining personality differences in rats," in *Handbook of Behavior Genetics*, ed K. Yong-Kyu (New York, NY: Springer), 281–300.

Driscoll, P., Ferré, P., Fernández-Teruel, A., Levi de Stein, M., Wolfman, C., Medina, J., et al. (1995). Effects of prenatal diazepam on two-way avoidance behavior, swimming navigation and brain levels of benzodiazepine-like molecules in male Roman High- and Low-Avoidance rats. *Psychopharmacology* 122, 51–57.

Ellenbroek, B., Geyer, M. A., and Cools, A. R. (1995). The behavior of APO-SUS rats in animal models with construct validity for schizophrenia. *J. Neurosci.* 15, 7604–7611.

Escorihuela, R. M., Fernández-Teruel, A., Gil, L., Aguilar, R., Tobeña, A., and Driscoll, P. (1999). Inbred roman high- and low-avoidance rats: differences in anxiety, novelty-seeking, and shuttlebox behaviors. *Physiol. Behav.* 67, 19–26. doi: 10.1016/S0031-9384(99)00064-5

Escorihuela, R. M., Tobeña, A., Driscoll, P., and Fernández-Teruel, A. (1995a). Effects of training, early handling, and perinatal flumazenil on shuttle box acquisition in Roman low-avoidance rats: toward overcoming a genetic deficit. *Neurosci. Biobehav. Rev.* 19, 353–367. doi: 10.1016/0149-7634(94)00051-2

Escorihuela, R. M., Tobena, A., and Fernández-Teruel, A. (1995b). Environmental enrichment and postnatal handling prevent spatial learning deficits in aged hypoemotional (Roman High-Avoidance) and hyperemotional (Roman Low-Avoidance) rats. *Learn. Mem.* 2, 40–48. doi: 10.1101/lm.2.1.40

Estanislau, C., Díaz-Morán, S., Cañete, T., Blázquez, G., Tobeña, A., and Fernández-Teruel, A. (2013). Context-dependent differences in grooming behavior among the NIH heterogeneous stock and the Roman high- and low-avoidance rats. *Neurosci. Res.* 77, 187–201. doi: 10.1016/j.neures.2013.09.012

Fernández-Teruel, A., Blázquez, G., Pérez, M., Aguilar, R., Canete, T., Guitart, M., et al. (2006). Latent inhibition threshold in roman high-avoidance rats: a psychogenetic model of abnormalities in attentional filter? *Actas Esp. Psiquiatr.* 34, 257–263.

Fernández-Teruel, A., Escorihuela, R. M., Castellano, B., Gonzalez, B., and Tobeña, A. (1997). Neonatal handling and environmental enrichment effects on emotionality, novelty/reward seeking, and age-related cognitive and hippocampal impairments: focus on the Roman rat lines. *Behav. Genet.* 27, 513–526. doi: 10.1023/A:1021400830503

Fernández-Teruel, A., Escorihuela, R. M., Núñez, J. F., Gomà, M., Driscoll, P., and Tobeña, A. (1992). Early stimulation effects on novelty-induced behavior in two psychogenetically-selected rat lines with divergent emotionality profiles. *Neurosci. Lett.* 137, 185–188. doi: 10.1016/0304-3940(92)90400-2

Freedman, R., Adler, L. E., Gerhardt, G. A., Waldo, M., Baker, N., Rose, G. M., et al. (1987). Neurobiological studies of sensory gating in schizophrenia. *Schizophr. Bull.* 13, 669–678.

Freudenberg, F., Dieckmann, M., Winter, S., Koch, M., and Schwabe, K. (2007). Selective breeding for deficient sensorimotor gating is accompanied by increased perseveration in rats. *Neuroscience* 148, 612–622. doi: 10.1016/j.neuroscience.2007.06.034

Garcia-Falgueras, A., Castillo-Ruiz, M. M., Put, T., Tobeña, A., and Fernández-Teruel, A. (2012). Differential hippocampal neuron density between inbred Roman high- (low anxious) and low-avoidance (high anxious) rats. *Neurosci. Lett.* 522, 41–46. doi: 10.1016/j.neulet.2012.06.011

García-Sánchez, F., Martínez-Gras, I., Rodríguez-Jiménez, R., and Rubio, G. (2011). Prepulse inhibition of the startle response/reflex in neuropsychiatric disorders. *Rev. Neurol.* 53, 422–432.

Giakoumaki, S. G., Bitsios, P., and Frangou, S. (2006). The level of prepulse inhibition in healthy individuals may index cortical modulation of early information processing. *Brain Res.* 1078, 168–170. doi: 10.1016/j.brainres.2006.01.056

Giakoumaki, S. G., Roussos, P., and Bitsios, P. (2008). Improvement of prepulse inhibition and executive function by the COMT inhibitor tolcapone depends on COMT Val158Met polymorphism. *Neuropsychopharmacology* 33, 3058–3068. doi: 10.1038/npp.2008.82

Giménez-Llort, L., Cañete, T., Guitart-Masip, M., Fernández-Teruel, A., and Tobeña, A. (2005). Two distinctive apomorphine-induced phenotypes in the Roman high- and low-avoidance rats. *Physiol. Behav.* 86, 458–466. doi: 10.1016/j.physbeh.2005.07.021

Giorgi, O., Lecca, D., Piras, G., Driscoll, P., and Corda, M. G. (2003). Dissociation between mesocortical dopamine release and fear-related behaviours in two psychogenetically selected lines of rats that differ in coping strategies to aversive conditions. *Eur. J. Neurosci.* 17, 2716–2726. doi: 10.1046/j.1460-9568.2003.02689.x

Giorgi, O., Piras, G., and Corda, M. G. (2007). The psychogenetically selected Roman high- and low-avoidance rat lines: a model to study the individual

vulnerability to drug addiction. *Neurosci. Biobehav. Rev.* 31, 148–163. doi: 10.1016/j.neubiorev.2006.07.008

Gomez, M. J., Escarabajal, M. D., de la Torre, L., Tobena, A., Fernández-Teruel, A., and Torres, C. (2009). Consummatory successive negative and anticipatory contrast effects in inbred Roman rats. *Physiol. Behav.* 97, 374–380. doi: 10.1016/j.physbeh.2009.03.003

Gray, J. A., Feldon, J., Rawlins, J. N. P., Hemsley, D. R., and Smith, A. D. (1991). The neuropsychology of schizophrenia. *Behav. Brain Res.* 14, 1–81.

Guitart-Masip, M., Johansson, B., Cañete, T., Fernández-Teruel, A., Tobeña, A., Terenius, L., et al. (2008). Regional adaptations in PSD-95, NGFI-A and secretogranin gene transcripts related to vulnerability to behavioral sensitization to amphetamine in the Roman rat strains. *Neuroscience* 151, 195–208. doi: 10.1016/j.neuroscience.2007.09.072

Hagan, J. J., and Jones, D. N. (2005). Predicting drug efficacy for cognitive deficits in schizophrenia. *Schizophr. Bull.* 31, 830–853. doi: 10.1093/schbul/sbi058

Hansen, C., and Spuhler, K. (1984). Development of the national institutes of health genetically heterogeneous stock. *Alcohol. Clin. Exp. Res.* 8, 477–479.

Johannesson, M., Lopez-Aumatell, R., Stridh, P., Diez, M., Tuncel, J., Blázquez, G., et al. (2009). A resource for the simultaneous high-resolution mapping of multiple quantitative trait loci in rats: the NIH heterogeneous stock. *Genome Res.* 19, 150–158. doi: 10.1101/gr.081497.108

Jones, C. A., Watson, D. J. G., and Fone, K. C. F. (2011). Animal models of schizophrenia. *Brit. J. Pharmacol.* 164, 1162–1194. doi: 10.1111/j.1476-5381.2011.01386.x

Kekesi, G., Petrovszki, Z., Benedek, G., and Horvath, G. (2015). Sex-specific alterations in behavioral and cognitive functions in a "three hit" animal model of schizophrenia. *Behav. Brain Res.* 284, 85–93. doi: 10.1016/j.bbr.2015.02.015

Klein, A. B., Ultved, L., Adamsen, D., Santini, M. A., Tobeña, A., Fernández-Teruel, A., et al. (2014). 5-HT(2A) and mGlu2 receptor binding levels are related to differences in impulsive behavior in the Roman Low- (RLA) and High- (RHA) avoidance rat strains. *Neuroscience* 263, 36–45. doi: 10.1016/j.neuroscience.2013.12.063

Koch, M., and Schnitzler, H. U. (1997). The acoustic startle response in rats-circuits mediating evocation, inhibition and potentiation. *Behav. Brain Res.* 89, 35–49.

Kohl, S., Heekeren, K., Klosterkötter, J., and Kuhn, J. (2013). Prepulse inhibition in psychiatric disorders–apart from schizophrenia. *J. Psychiatr. Res.* 47, 445–452. doi: 10.1016/j.jpsychires.2012.11.018

López-Aumatell, R., Blázquez, G., Gil, L., Aguilar, R., Cañete, T., Giménez-Llort, L., et al. (2009c). The Roman High and Low-Avoidance rat strains differ in fear-potentiated startle and classical aversive conditioning. *Psicothema* 21, 27–32.

López-Aumatell, R., Guitart-Masip, M., Vicens-Costa, E., Gimenéz-Llort, L., Valdar, W., Johannesson, M., et al. (2008). Fearfulness in a large N/Nih genetically heterogeneous rat stock: differential profiles of timidity and defensive flight in males and females. *Behav. Brain Res.* 188, 41–55. doi: 10.1016/j.bbr.2007.10.015

López-Aumatell, R., Martínez-Membrives, E., Vicens-Costa, E., Cañete, T., Blázquez, G., Mont-Cardona, C., et al. (2011). Effects of environmental and physiological covariates on sex differences in unconditioned and conditioned anxiety and fear in a large sample of genetically heterogeneous (N/Nih-HS) rats. *Behav. Brain Funct.* 7:48. doi: 10.1186/1744-9081-7-48

López-Aumatell, R., Vicens-Costa, E., Guitart-Masip, M., Martínez-Membrives, E., Valdar, W., Johannesson, M., et al. (2009a). Unlearned anxiety predicts learned fear: a comparison among heterogeneous rats and the Roman rat strains. *Behav. Brain Res.* 202, 92–101. doi: 10.1016/j.bbr.2009.03.024

López-Aumatell, R., Vicens-Costa, E., Guitart-Masip, M., Martínez-Membrives, E., Valdar, W., Johannesson, M., et al. (2009b). Ansiedad en ratas genéticamente heterogéneas: hacia la identificación de genes para caracteres conductuales cuantitativos. *Ansiedad Estrés.* 15, 67–84.

Manzo, L., Gómez, M. A. J., Callejas-Aguilera, J. E., Donaire, R., Sabariego, M., Fernández-Teruel, A., et al. (2014). Relationship between ethanol preference and sensation/novelty seeking. *Physiol. Behav.* 133, 53–60. doi: 10.1016/j.physbeh.2014.05.003

Martínez-Membrives, E., López-Aumatell, R., Blázquez, G., Cañete, T., Tobeña, A., and Fernández-Teruel, A. (2015). Spatial learning in the genetically heterogeneous NIH-HS rat stock and RLA-I/RHA-I rats: revisiting the relationship with unconditioned and conditioned anxiety. *Physiol. Behav.* 144, 15–25. doi: 10.1016/j.physbeh.2015.03.003

Meyza, K. Z., Boguszewski, P. M., Nikolaev, E., and Zagrodzka, J. (2009). Diverse sensitivity of RHA/Verh and RLA/Verh rats to emotional and spatial aspects of a novel environment as a result of a distinct pattern of neuronal activation in the fear/anxiety circuit. *Behav. Genet.* 39, 48–61. doi: 10.1007/s10519-008-9234-z

Moreno, M., Cardona, D., Gómez, M. J., Sánchez-Santed, F., Tobeña, A., Fernández-Teruel, A., et al. (2010). Impulsivity characterization in the Roman high- and low-avoidance rat strains: behavioral and neurochemical differences. *Neuropsychopharmacology* 35, 1198–1208. doi: 10.1038/npp.2009.224

Morris, R. G. M., Anderson, E., Lynch, G. S., and Baudry, M. (1986b). Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartat receptor antagonist, AP5. *Nature* 319, 774–776.

Morris, R. G. M., Hagan, J. J., and Rawlins, J. N. P. (1986a). Allocentric spatial learning by hippocampectomized rats: a further test of the "spatial mapping" and "working memory" theories of hippocampal function. *Q. J. Exp. Psychol.* 38B, 365–395.

Nil, R., and Bättig, K. (1981). Spontaneous maze ambulation and Hebb-Williams learning in Roman High-Avoidance and Roman Low-Avoidance rats. *Behav. Neural Biol.* 33, 465–475. doi: 10.1016/S0163-1047(81)91833-1

Palència, M., Díaz-Morán, S., Mont-Cardona, C., Cañete, T., Blázquez, G., Martínez-Membrives, E. et al. (2013). Helplessness-like escape deficits of NIH-HS rats predict passive behavior in the forced swimming test: relevance for the concurrent validity of rat models of depression. *World J. Neurosci.* 3, 83–92. doi: 10.4236/wjns.2013.32012

Petrovszki, Z., Adam, G., Tuboly, G., Kekesi, G., Benedek, G., Keri, S., et al. (2013). Characterization of gene-environment interactions by behavioral profiling of selectively bred rats: the effect of NMDA receptor inhibition and social isolation. *Behav. Brain Res.* 240, 134–145. doi: 10.1016/j.bbr.2012.11.022

Powell, C. M., and Miyakawa, T. (2006). Schizophrenia-relevant behavioral testing in rodent models: a uniquely human disorder? *Biol. Psychiatry* 59, 1198–1207. doi: 10.1016/j.biopsych.2006.05.008

Rosas, J. M., Callejas-Aguilera, J. E., Escarabajal, M. D., Gomez, M. J., de la Torre, L., Agüero, A., et al. (2007). Successive negative contrast effect in instrumental runway behaviour: a study with Roman high- (RHA) and Roman low- (RLA) avoidance rats. *Behav. Brain Res.* 185, 1–8. doi: 10.1016/j.bbr.2007.07.027

Sallés, J., López de Jesús, M., Goñi, O., Fernández-Teruel, A., Driscoll, P., Tobeña, A., et al. (2001). Transmembrane signaling through phospholipase c in cortical and hippocampal membranes of psychogenetically selected rat lines. *Psychopharmacology* 154, 115–125. doi: 10.1007/s002130000621

Sawa, A., and Snyder, S. (2002). Schizophrenia: diverse approaches to a complex disease. *Science* 296, 692–695. doi: 10.1126/science.1070532

Schwabe, K., Freudenberg, F., and Koch, M. (2007). Selective breeding of reduced sensorimotor gating in Wistar rats. *Behav. Genet.* 37, 706–712. doi: 10.1007/s10519-007-9166-z

Singer, P., Hauser, J., Llano Lopez, L., Peleg-Raibstein, D., Feldon, J., Gargiulo, P. A., et al. (2013). Prepulse inhibition predicts working memory performance whilst startle habituation predicts spatial reference memory retention in C57BL/6 mice. *Behav. Brain Res.* 242, 166–177. doi: 10.1016/j.bbr.2012.12.011

Spuhler, K., and Deitrich, R. A. (1984). Correlative analysis of ethanol-related phenotypes in rat inbred strains. *Alcohol. Clin. Exp. Res.* 8, 480–484.

Steimer, T., and Driscoll, P. (2003). Divergent stress responses and coping styles in psychogenetically selected Roman High-(RHA) and Low-(RLA) avoidance rats: behavioural, neuroendocrine and developmental aspects. *Stress* 6, 87–100. doi: 10.1080/1025389031000111320

Steimer, T., and Driscoll, P. (2005). Inter-individual vs line/strain differences in psychogenetically selected Roman High-(RHA) and Low-(RLA) avoidance rats: neuroendocrine and behavioural aspects. *Neurosci. Biobehav. Rev.* 29, 99–112. doi: 10.1016/j.neubiorev.2004.07.002

Torres, C., Candido, A., Escarabajal, M. D., de la Torre, L., Maldonado, A., Tobeña, A., et al. (2005). Successive negative contrast in one-way avoidance learning in female Roman rats. *Physiol. Behav.* 85, 377–382. doi: 10.1016/j.physbeh.2005.02.023

Tsaih, S. W., Holl, K., Jia, S., Kaldunski, M., Tschannen, M., He, H., et al. (2014). Identification of a novel gene for diabetic traits in rats, mice, and humans. *Genetics* 198, 17–29. doi: 10.1534/genetics.114.162982

van der Elst, M. C. J., Ellenbroek, B. A., and Cools, A. R. (2006). Cocaine strongly reduces prepulse inhibition in apomorphine-susceptible rats, but not in apomorphine-unsusceptible rats: regulation by dopamine D2 receptors. *Behav. Brain Res.* 175, 392–398. doi: 10.1016/j.bbr.2006.09.014

Vicens-Costa, E., Martínez-Membrives, E., López-Aumatell, R., Guitart-Masip, M., Cañete, T., Blázquez, G., et al. (2011). Two-way avoidance acquisition is negatively related to conditioned freezing and positively associated with startle reactions: a dissection of anxiety and fear in genetically heterogeneous rats. *Physiol. Behav.* 103, 148–156. doi: 10.1016/j.physbeh.2010.12.009

Whishaw, I. Q. (1985). Formation of a place learning-set by the rat: a new paradigm for neurobehavioral studies. *Physiol. Behav.* 35, 139–143.

Wible, C. G. (2013). Hippocampal physiology, structure and function and the neuroscience of schizophrenia: a unified account of declarative memory deficits, working memory deficits and schizophrenic symptoms. *Behav. Sci. (Basel.)* 3, 298–315. doi: 10.3390/bs3020298

Young, J. W., Powell, S. B., Risbrough, V., Marston, H. M., and Geyer, M. A. (2009). Using the MATRICS to guide development of a preclinical cognitive test battery for research in schizophrenia. *Pharmacol. Therapeut.* 122, 150–202. doi: 10.1016/j.pharmthera.2009.02.004

Zeier, H., Baettig, K., and Driscoll, P. (1978). Acquisition of DRL-20 behavior in male and female, Roman high- and low-avoidance rats. *Physiol. Behav.* 20, 791–793. doi: 10.1016/0031-9384(78)90307-4

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

10. ESTUDI 2:

Divergent effects of isolation rearing on prepulse inhibition, activity, anxiety and hippocampal-dependent memory in Roman high- and low-avoidance rats: A putative model of schizophrenia-relevant features

Behavioural Brain Research 314 (2016) 6–15

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Divergent effects of isolation rearing on prepulse inhibition, activity, anxiety and hippocampal-dependent memory in Roman high- and low-avoidance rats: A putative model of schizophrenia-relevant features

Ignasi Oliveras[a,*], Ana Sánchez-González[a], Maria Antonietta Piludu[b], Cristina Gerboles[a], Cristóbal Río-Álamos[a], Adolf Tobeña[a], Alberto Fernández-Teruel[a,*]

[a] Medical Psychology Unit, Department of Psychiatry and Forensic Medicine, School of Medicine, Institute of Neurosciences, Autonomous University of Barcelona 08193-Bellaterra, Barcelona, Spain
[b] Department of Life and Environmental Sciences, Section of Pharmaceutical, Pharmacological and Nutraceutical Sciences, University of Cagliari, 09124 Cagliari, Italy

HIGHLIGHTS

- Isolation rearing induces differential effects in RHA-I and RLA-I rats.
- Isolation rearing induces PPI deficits, hyperactivity and anxiety in RHA-I rats.
- Isolation rearing induces spatial reference memory deficits in RHA-I rats.
- Isolation rearing induces spatial working memory deficits in RLA-I rats.
- RHA-I rats may be a valid model for schizophrenia-relevant features.

ARTICLE INFO

Article history:
Received 25 May 2016
Received in revised form 25 July 2016
Accepted 28 July 2016
Available online 28 July 2016

Keywords:
Roman high- and low-avoidance rats
Genetically-based rat model
Schizophrenia
Isolation rearing
Prepulse inhibition
Spatial learning/memory

ABSTRACT

Social isolation of rats induces a constellation of behavioral alterations known as "isolation syndrome" that are consistent **with** some of the positive and cognitive symptoms observed in schizophrenic patients. In the present study we have assessed whether isolation rearing of inbred Roman high-avoidance (RHA-I) and Roman low-avoidance (RLA-I) strains can lead to the appearance of some of the key features of the "isolation syndrome", such as prepulse inhibition (PPI) deficits, increased anxious behavior, hyperactivity and memory/learning impairments. Compared to RLA-I rats, the results show that isolation rearing (IR) in RHA-I rats has a more profound impact, as they exhibit isolation-induced PPI deficits, increased anxiety, hyperactivity and long-term reference memory deficits, while isolated RLA-I rats only exhibit deficits in a spatial working memory task. These results give further support to the validity of RHA-I rats as a genetically-based model of schizophrenia relevant-symptoms.

1. Introduction

Isolation rearing (IR) has been used to induce detrimental effects on brain development and adult behavior which mimic some features or symptoms present in neurodevelopmental disorders, including schizophrenia. The alterations induced by IR are enduring, robust and replicable, which may allow a better understanding of the neurobiological disturbances causing a wide range of symptoms relevant to developmental psychiatric disorders like schizophrenia. This will also facilitate the discovery of new pharmacological targets with enhanced efficacy and less undesired effects [1]

A variety of behavioral abnormalities induced by IR have been reported (for a review see Ref. [1]). These behavioral changes are usually labeled as "isolation syndrome" and, according to Jones et al. [2] and Fone and Porkess [1] they include hyperactivity, neophobia,

Abbreviations: IR, Isolation rearing; RHA-I, Roman high-avoidance rats; RLA-I, Roman low-avoidance rats; PPI, Prepulse inhibition; MWM, Morris water maze.

* Corresponding author.
E-mail addresses: Ignasi.oliveras@uab.cat (I. Oliveras), Albert.fernandez.teruel@uab.cat (A. Fernández-Teruel).

http://dx.doi.org/10.1016/j.bbr.2016.07.047
0166-4328/

prepulse inhibition (PPI) deficits, cognitive/learning impairments and increased anxious and aggressive behaviors [1,3,4,5–7].

The Roman high-(RHA) and low-avoidance (RLA) rat lines/strains (depending on whether they are outbred – i.e., lines-, or inbred – i.e., strains-) have been selectively and bidirectionally bred for their rapid (RHA) vs. extremely poor (RLA) ability to acquire the two-way active avoidance task [8,9]. Extensive research conducted over near four decades has revealed that anxiety/fear and stress sensitivity are among the most prominent behavioral traits that distinguish the two Roman lines/strains [for review see Refs. 8,9]. Thus, compared to their RLA counterparts, RHA rats (both from the outbred line – RHA/Verh- or from the inbred strain – RHA-I-) show decreased unconditioned and conditioned anxiety/fear [e.g. Refs. 8,9,10,11,12,13], a proactive coping style [e.g. Refs. 13,14] and lowered activation of the hypothalamus–pituitary–adrenal (HPA) axis in response to stress [e.g. Refs. 13,14,15].

In Del Rio et al. [16] and Oliveras et al. [17] we proposed that the Roman high- and low-avoidance rat lines/strains could be a valid model of differential schizophrenia-related features. Thus, compared to RLA rats, RHAs show enhanced impulsive behavior in the 5-choice serial reaction time (5-CSRTT) and DRL-20 operant tasks [18–20], deficits in latent inhibition (in this case RHA-I rats were compared with Sprague Dawley rats[21]; and unpublished results from our laboratory), and impaired PPI and spatial working and reference memory [16,17,22,23–25]. Regarding the predictive and construct validity of the model, RHA rats show enhanced locomotor activity as well as mesolimbic dopaminergic sensitization to repeated (DAergic) psychostimulant administration [26–28], augmented mesocortical dopaminergic response to stress [29], increased stereotypic response to the dopamine agonist apomorphine [30–32] and neurochemical and neuromorphological evidence of decreased hippocampal function [33–35]. Furthermore, Klein et al. [20] showed that RHA-I had no detectable expression of mGluR2 in the frontal cortex, hippocampus and striatum, as well as increased fronto-cortical density of 5-HT2A receptors (for the implications of the mGluR2 in schizophrenia see Ref. [36]). Collectively these RHA vs RLA profiles suggest that (inbred and outbred) RHA rats may be a valid model of some behavioral and neurobiological features related with schizophrenia.

In the present study we evaluated the impact that IR has on prepulse inhibition (PPI) of startle, on anxiety in the elevated zero-maze, on locomotor activity in a new environment and on spatial working memory and long-term reference memory in the Morris water maze (MWM). We hypothesized that RHA-I rats will exhibit more profound and robust IR-induced deficits in these tasks than their RLA-I counterparts.

2. Methods

2.1. Subjects and housing

At postnatal day 21, male RHA-I rats (n = 36) and RLA-I (n = 40) were randomly assigned to one of the two housing conditions: in pairs (12 RHA-I rats and 16 RLA-I rats; in macrolon cages of 50 cm × 25 cm × 15 cm) or isolated (24 rats of each strain; in macrolon cages of 35 cm × 25 cm × 15 cm). Isolated rats had auditory, olfactory and visual but not physical contact with littermates. Rats from each experimental group came from 7 to 8 different litters. We included a greater number of rats in the isolated groups because preliminary PPI experiments (data not shown) suggested us that there could be larger variability as a consequence of IR treatment, and thus the increase in the number of animals in the IR groups might eventually reduce variability. All rats were reared and maintained in the same animal room, under a 12:12 h light-dark cycle (lights on at 08:00 a.m.), with controlled temperature (22 ± 2 °C) and humidity (50–70%) and with free access to food and water. After 14 weeks of isolation the experimental procedures began as described below, and the housing conditions were the same throughout the whole test battery. All experiments/tests were carried out during the light phase of the cycle. The experiments were approved by the committee of Ethics of the Autonomous University of Barcelona in accordance with the European Communities Council Directive (86/609/EEC) regarding the care and use of animals for experimental procedures.

2.2. PPI

After 14 weeks of isolation rats were submitted to PPI testing. Four startle boxes (SR-Lab Startle Response System, San Diego Inst., San Diego, USA) were used. They consist in a Plexiglas cylinder situated on the top of a platform with a sensor that detects changes in strength made by the movements of the rat in each trial. Auditory stimuli were delivered by two speakers situated 15 cm from each side of the cylinder. Each box is constantly lit by a 10 W lamp. The data are transduced by an accelerometer into a voltage and then saved into a computer for further analysis.

The startle session started with a 5 min habituation period in the startle chambers. Then, 10 “pulse-alone” trials (105 dB, 40 ms) are delivered in order to obtain a baseline measure of the startle response (Baseline 1). After this, each one of the 4 different types of trials (pulse-alone, prepulse-pulse and no stimulus trials) were randomly administered 10 times (40 trials in total): Pulse-alone trials (105 dB, 40 ms, Baseline 2, this was the variable used to calculate the %PPI; see the equation below); Prepulses of 59 dB and 63 dB (20 ms) followed by the pulse (105 dB, 40 ms), with an interstimulus interval of 100 ms; finally, 10 trials in which no stimulus was delivered and only the background noise was present (55 dB). At the end of the session, 5 “pulse-alone” trials were delivered to have a measure of habituation to the startle stimulus (Baseline 3). The interval between trials was 10–20 s with a mean of 15s. The maximal magnitude (i.e. the peak) of startle response was recorded during 200 ms after the onset of the pulse.

The percentage of PPI (%PPI) is calculated according to the formula: %PPI = 100 − ((startle amplitude on prepulse trials/startle amplitude on pulse trials) × 100)

2.3. Elevated zero-maze

Two weeks later (16 weeks of isolation), rats were tested in the elevated zero-maze. The maze, similar to that described by Shepherd et al. [37], is composed of an annular platform (i.e., a circular corridor 10 cm width and 105 cm diameter). The maze is made of black plywood and is elevated 65 cm above the ground level. It has two open sections (quadrants) and two enclosed ones (with walls 40 cm height).

The maze was placed in a room dimly illuminated by a red light. The trials were videotaped and the measures were taken outside the testing room by a trained observer. Trials began with the animals placed in the enclosed quadrant facing the wall.

The following variables were measured for 5 min (see Refs. [38,10]): time spent in open sections, number of entries into open sections, latency of the first entry to the open section, head dips through the edge of the open sections of the maze and line crossings among the eight different zones defined in the maze.

2.4. Locomotor activity

Two weeks later (18 weeks of isolation), rats were tested in the activity boxes to assess horizontal locomotor activity. The test was carried out in 3 identical Plexiglas activity cages (40 × 40 × 40 cm).

These cages are equipped with a frame of photocell beams that are interfaced by computer software (Acti-Track; PANLAB, Barcelona, Spain), in order to record the horizontal behavior of the rats. The test began with the rat in the center of the box and lasted for 30 min. The testing room was illuminated with a fluorescent light (60 W). Locomotor activity was measured as the total photocell beam breaks in 30 min.

2.5. Morris water maze

Finally, 2 weeks after assessment of activity (20 weeks of isolation), rats were evaluated in 4 different tasks in the Morris water maze. The testing apparatus consisted of a circular pool (diameter: 150 cm, height: 60 cm), filled to a depth of 30 cm with 23 ± 1 °C water made opaque with white paint. There were no local cues available in the swimming pool.

Four points and quadrants that were equally spaced around the perimeter of the tank were arbitrarily designed to serve as starting positions and platform (diameter: 15 cm, height: 27 cm) locations. The starting positions and the platform locations were as follows: N, S, E, W and NE, NW, SE, SW, respectively.

The behavior of the animal (latency, distance and speed) was monitored by a video camera mounted on the ceiling above the center of the pool and using a tracking system (Smart v.2.5.14; PANLAB, Barcelona, Spain).

2.5.1. Delayed matching to place (DMTP) task

On 4 consecutive days the animals were allowed to swim for 90 s or until they located a platform submerged 2 cm in a fixed position each day. Each rat went through 2 trials per day: a sample/acquisition trial and a retention trial. If a rat did not find the platform in 90 s, it was gently guided to it by the experimenter. Rats were allowed to stay for 15 s on the platform and then they spent another 15 s in an individual cage before the next trial started. Intertrial interval was 30 s.

Four platform positions were defined: the first day the platform was located in the center of the NW quadrant, the second day it was located at a distance of 15 cm in the S direction and the third day the platform was at the center of the tank. And finally on the fourth day it was located in the center of the SE quadrant. Four starting positions were also defined: E, W, S and N respectively. The starting point and the location of the platform were pseudo-randomly varied each day. Several room cues were constantly visible from the pool.

Escape latencies, path lengths, and swimming speed from each rat and trial were provided by a tracking system (Smart v.2.5.14; PANLAB, Barcelona, Spain) connected to a video camera placed above the pool.

2.5.2. Place learning and transfer test

2.5.2.1. Place task. The training session consisted of 3 trials/day during 4 consecutive days (12 trials). Each trial started from one of the four starting positions. The order of starting positions (N, S, E or W) was randomly determined. A trial began by placing the rat into the water facing the wall of the pool at one of the starting points, and if the rat failed to escape within 90s, it was gently guided to the platform by the experimenter. Once the rat reached the platform (located in the center of the SW quadrant), it was allowed to stay there for 15s. Approximately 25–30 min elapsed between consecutive trials. The parameter used in this study was the distance travelled to reach the submerged platform, as differences in navigation speed among the different experimental groups were observed.

2.5.2.2. Transfer test. Recall of the platform location was tested 3 h later over 60 s in a test in which the platform had been removed. The trial started from the "S" point with the rats facing the wall. The parameters measured in this task were (a) latency to reach the platform position, (b) distance travelled and (c) number of entries into the platform position (annulus) and (d) the permanence time in the annulus.

2.5.3. Cued task

2.5.3.1. Cued task. Consisted of 4 consecutive trials (with four different starting positions: N, S, E and W) administered 7–8 days after the transfer test. Approximately 25 min elapsed between consecutive trials. For this test, the platform was visible (1 cm above the water surface) and cued with a small striped (dark blue and white) flag and placed in the NE quadrant. The distance travelled to reach the platform was used to assess the performance in this task.

2.6. Analysis

Statistical analysis was performed using the "Statistical Package for Social Science" (SPSS, version 17).

To analyze results, two-way ANOVAs with strain (RHA-I and RLA-I) and IR treatment (isolation or control) as between-subject factors were applied to all "total" test measures.

For PPI data, a repeated measures ANOVA was applied with strain and IR treatment as between-subject factors and the prepulse intensities (59 dB and 63 dB) as within-subject factor. For activity results a repeated measures ANOVA was performed with the time intervals (6 interval of 5 min each) as a within-subject factor and strain and IR treatment as between-subject factor. In the DMTP task a repeated measures ANOVA was also performed with the 2 trials as a within-subject factor and the strain and IR treatment as between-subject factors. To analyze place-learning a repeated measures ANOVA was conducted with strain and IR treatment as between-subject factors and the 12 trials (3 trials for 4 days) as a within-subject factor. Finally a repeated measures ANOVA was conducted for the cued task, with strain and IR treatment as between-subject factors and the 4 trials as within-subject factor.

Further analysis with one-way ANOVAs within each strain were applied when the previous overall ANOVAs led to significant "strain × treatment", "strain × time interval" or "strain × trial" interaction effects. There were only three exceptions in which the initial overall ANOVA did not show interaction effects (entries in open sections and line crossings in the elevated zero-maze, and total distance travelled in the motor activity test), but separated one-way ANOVAs within each strain were nevertheless applied because it was expected that the IR treatment would not affect both strains with the same magnitude (or direction).

In the delayed matching to place task a logarithm transformation of the distances travelled in the first and second trials was used in order to reduce the variability. The difference between the first and second trial was built from this transformation.

3. Results

3.1. PPI

Repeated measures ANOVA with strain (RHA-I or RLA-I) and treatment (isolated or grouped) as between-subject factors and the prepulse intensities (59 dB and 63 dB) as within-subject factor revealed a significant "strain × treatment" interaction ($F(1, 72) = 4.71$ $p = 0.033$) and a significant "strain" effect ($F(1, 72) = 8.82$ $p = 0.004$) (Fig. 1A,C). Further one-way ANOVAs for each strain and prepulse intensity revealed a significant "treatment" effect in isolated RHA-I rats ($F(1, 34) = 5.62$ $p = 0.024$) at the 59 dB prepulse intensity, indicating that IR reduced significantly the %PPI of the

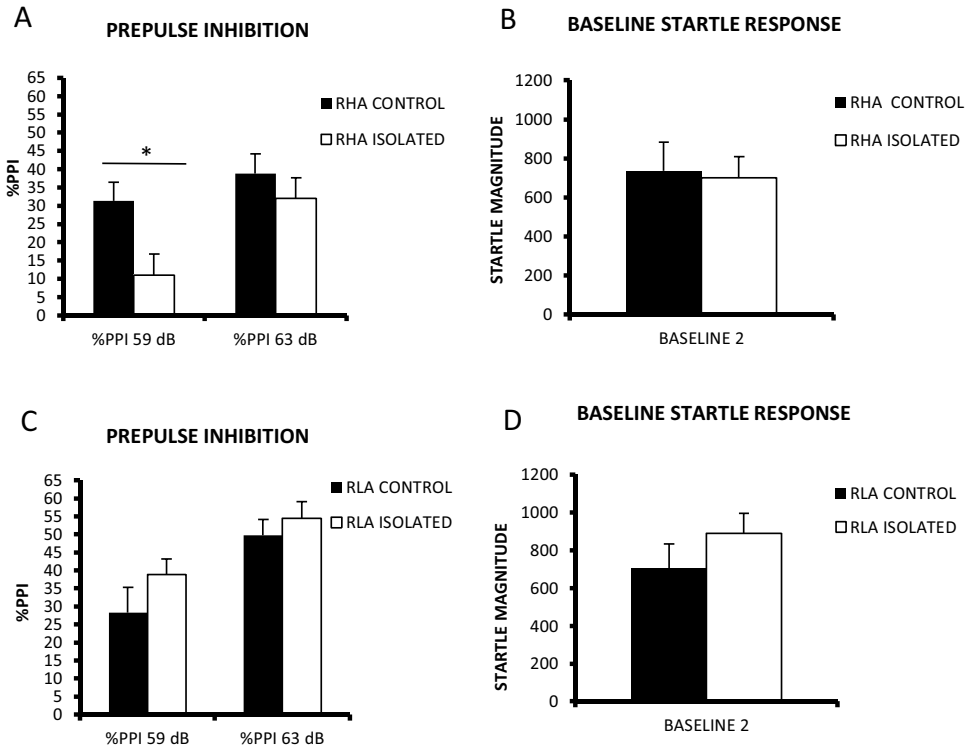


Fig. 1. (A) Mean ± S.E.M.%PPI for RHA-I rats in the two prepulse intensities tested (59 dB and 63 dB). (B) Mean ± S.E.M. of baseline startle response of RHA-I rats in the 10 pulse-alone trials during the "Baseline 2" phase (see "Methods"). (C) Mean ± S.E.M.%PPI of RLA-I rats. D) Mean ± S.E.M. of baseline startle response of RLA-I rats in the 10 pulse-alone trials during the "Baseline 2" phase (see "Methods"). *p < 0.05, one-way ANOVA following significant overall ANOVA.

RHA-I strain, while there were no significant differences between both RLA-I groups ($p > 0.17$) (Fig. 1A,C).

ANOVA on baseline startle response (Fig. 1B and D) did not reveal any significant effect of the strain or the treatment.

3.2. Elevated zero-maze

ANOVA with strain and treatment as between-subject factors on the "latency of entry to the open sections" revealed a significant "strain × treatment" interaction ($F(1, 72) = 4.00$ $p = 0.049$). Separate ANOVAs for each strain showed no differences between treatments in RLA-I rats and a significant effect of isolation in the RHA-I strain ($F(1, 34) = 5.05$ $p < 0.031$), indicating that isolation-reared rats delayed their entry to the open spaces of the maze (Fig. 2A).

Similarly, a significant "strain" × "treatment" interaction ($F(1, 72) = 10.20$ $p = 0.002$) was observed for "time spent in the open sections", and the ANOVAs for each strain also revealed no isolation effect in RLA-I rats, while for the RHA-I rats a significant treatment effect ($F(1,34) = 15.49$ $p < 0.001$) was found, indicating that isolated RHA-I rats spent significantly shorter time in the open spaces compared to the control group (Fig. 2B).

The number of entries into the open sections showed a significant "strain" effect ($F(1, 72) = 11.37$ $p = 0.001$). No treatment effect was found in RLA-I rats. In the RHA-Is, isolation had a significant effect ($F(1, 34) = 4.17$ $p = 0.05$) (Fig. 2C).

Two-way ANOVA of head dips showed a significant "strain × treatment" interaction ($F(1, 72) = 19.71$ $p < 0.001$). Separate ANOVAs for each strain revealed a significant effect of isolation in the RHA-Is ($F(1, 34) = 60.38$ $p < 0.001$) (Fig. 2D).

Two-way ANOVA of line crossings revealed a significant "strain" effect ($F(1, 72) = 10.27$ $p = 0.002$), but separated ANOVAs for each strain did not show any effect of isolation treatment (Fig. 2E).

3.3. Horizontal locomotor activity in the activity cages

Repeated measures ANOVA, with strain and treatment as between-subject factors and six time intervals (of 5 min each) as a within-subject factor, revealed a significant "strain × interval" interaction ($F(5,360) = 2.73$ $p = 0.020$) (Fig. 3A and C). The two main effects were also significant: $F(1, 72) = 7.21$ $p = 0.009$ for the strain effect and $F(1, 72) = 4.58$ $p = 0.036$ for the treatment effect (Fig. 3A and C). A repeated measures ANOVA for each strain revealed a significant treatment effect in RHA-I rats ($F(1, 32) = 8.89$ $p = 0.005$), indicating that RHA-I rats reared in isolation travelled longer distances during the 30-min session (Fig. 3A). ANOVAs for each interval were also performed, and the results show that in 4 out of 6 (1st, 3rd, 5th and 6th) intervals there were significant differences between both RHA-I groups, as isolated RHA-I rats travelled longer distances (all $Fs(1, 34) \geq 4.34$ $p \leq 0.045$) (Fig. 3A).

A two-way ANOVA for the "total distance travelled" revealed significant "strain" and "treatment" effects ($F(1, 72) = 7.21$ $p = 0.009$ and $F(1, 72) = 4.58$ $p = 0.036$, respectively; Fig. 3B and 3D). Further one-way ANOVAs for each strain showed that only RHA-I groups are significantly different in that variable (Fig. 3B).

3.4. Morris water maze

3.4.1. Delayed matching to place task (DMPT)

Repeated measures ANOVA on the mean distance travelled in the first trials and the mean distance travelled in the second trials yielded a significant "trial × strain" interaction ($F(1,72) = 11.61$ $p = 0.001$) (Fig. 4A). The two main effects were also significant: strain ($F(1, 72) = 32.70$ $p < 0.001$) and treatment ($F(1, 72) = 5.74$ $p = 0.019$). Repeated measures ANOVA for each strain revealed a significant interaction between trial and treatment ($F(1, 38) = 4.69$ $p = 0.037$) and a significant effect of isolation only in RLA-I rats ($F(1, 38) = 5.21$ $p = 0.027$). Separated ANOVAs for each trial revealed no

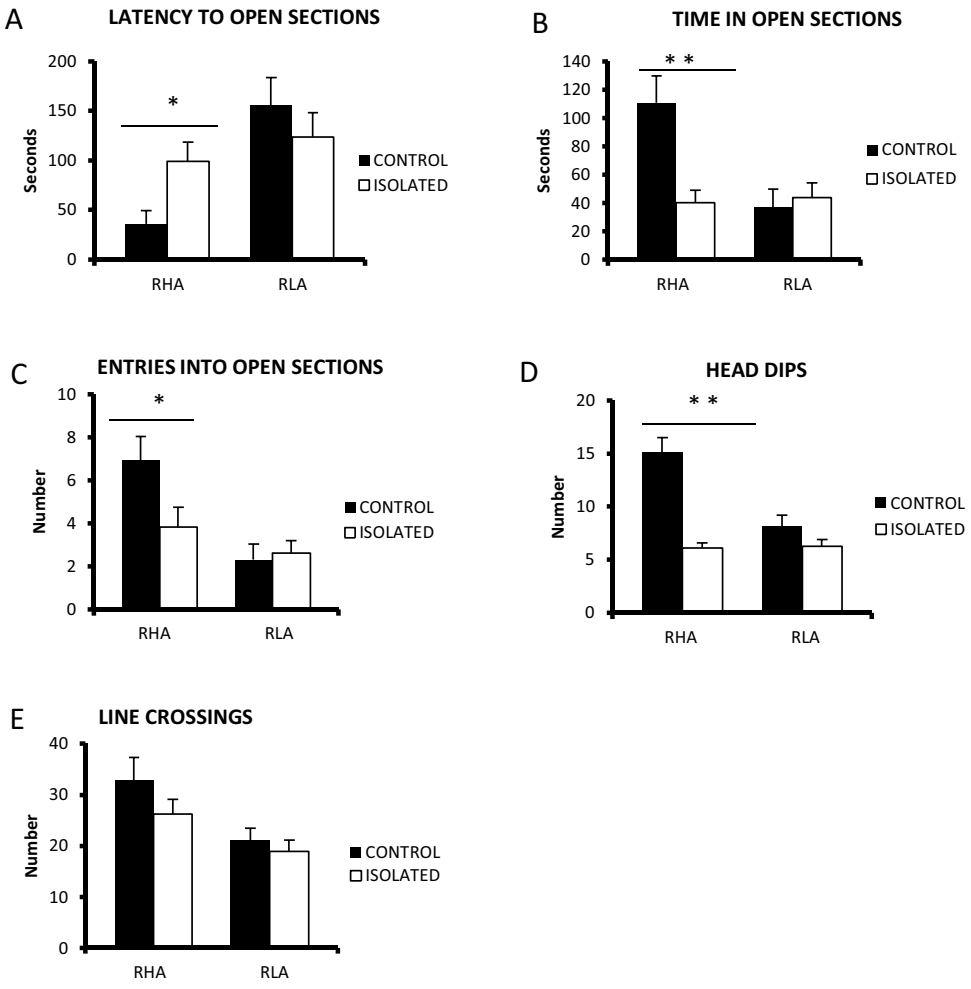


Fig. 2. Mean ± S.E.M. of (A) latency to (the first entry into) open sections; (B) time (spent) in open sections; (C) entries into open sections; (D) head dips and E) line crossings, for both rat strains and treatments. * $p \leq 0.05$, ** $p < 0.01$, one-way ANOVA following significant overall ANOVA.

significant differences between groups ($p = 0.338$) in the first trial, whereas in the second trial there were significant differences ($F(1, 38) = 7.67$ $p = 0.009$; Fig. 4A) indicating that isolated RLA-I rats travelled longer distances than RLA-I controls.

Two-way analysis of covariance (ANCOVA) of the mean distance difference between the first and second trials, taking the distance travelled in the first trials as a covariate, revealed significant main effects of the strain ($F(1,71) = 28.72$ $p < 0.001$) and the treatment ($F(1,71) = 6.36$ $p = 0.014$). ANOVAs for each strain revealed a significant effect of isolation rearing in RLA-I rats ($F(1, 38) = 4.19$ $p = 0.048$) (Fig. 4B).

Together, the previous analyses indicate that isolation rearing induced a working memory deficit in the RLA-I rats while there was no IR effect in the RHA-I rats.

3.4.2. Place learning

Repeated measures ANOVA of the distance travelled in each trial (3 trials/day) did not reveal any significant effect (only a tendency to significance of the treatment, $p = 0.075$) (Fig. 5A, B).

3.4.3. Transfer test

A two-way ANOVA on annulus crossings (i.e. crossings of the annulus where the platform was located during the place learning task) was performed with strain and treatment as between-subject factors. This analysis yielded a significant "strain × treatment" interaction ($F(1, 72) = 6.85$ $p = 0.011$) (Fig. 6A). Further ANOVAs for each strain on the same variable showed a significant effect of isolation in RHA-I rats ($F(1, 34) = 11.92$ $p = 0.001$), meaning that isolated RHA-I made less annulus crossings (Fig. 6A). We also conducted the same analysis for the permanence time in the annulus and we found a "treatment" effect ($F(1, 72) = 5.03$ $p = 0.028$) (Fig. 6B). This effect was detected only in RHA-I rats ($F(1, 34) = 10.70$ $p = 0.002$) when we performed separate ANOVAs for each strain. This also suggests that isolation rearing worsened long term reference memory only in RHA-I rats.

3.4.4. Cue task

Repeated measures ANCOVA on the distance travelled in trials 2–4, taking the distance travelled in the first trial as a covariate, was performed. The results did not reveal any effect of the strain or the treatment, indicating that no gross motivational, motor or visual problems were present in any of the groups (Fig. 7).

4. Discussion

In the present study we demonstrate that IR has severe effects in the Roman rats in tests/tasks that measure processes linked to schizophrenia-related symptoms and anxiety. Isolation rearing induced a significant impairment of PPI in RHA-I rats, as well as a marked increase of anxiety in the elevated zero-maze, whereas the treatment did not affect the RLA-I strain in these tests. In the activity cages we found that RHA-I rats reared in isolation travelled

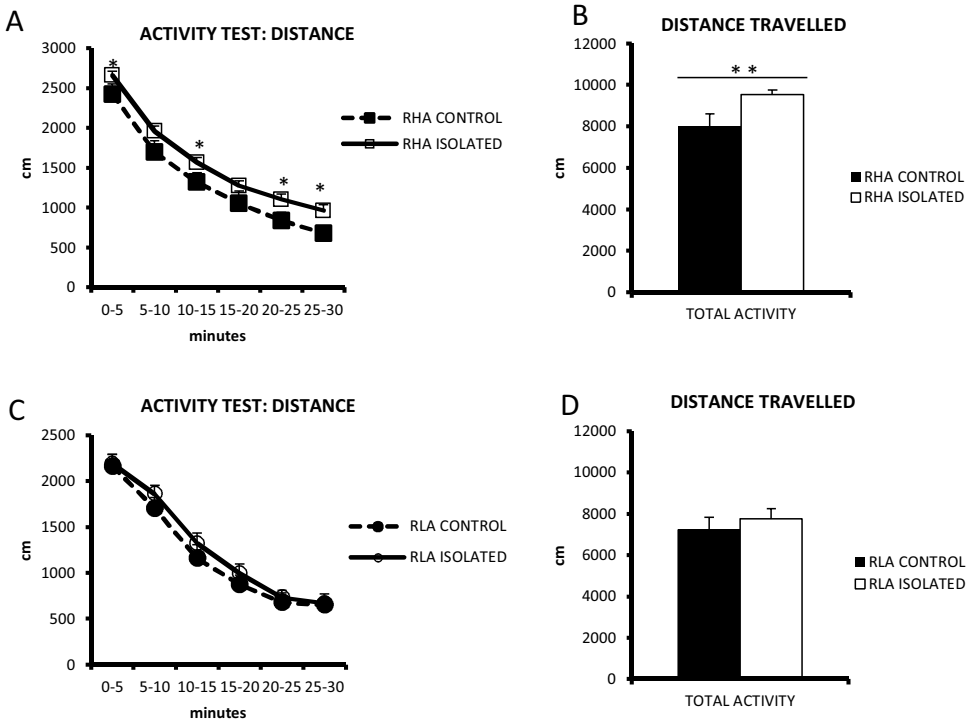


Fig. 3. (A) Mean ± S.E.M. distances of the RHA-I (control and isolated) rats divided in 5-min intervals; (B) Mean ± S.E.M. of the total distance travelled by RHA-I (control and isolated) rats. (C) Mean ± S.E.M. of distances of the RLA-I (control and isolated) rats divided in 5-min intervals; (D) Mean ± S.E.M. of the total distance travelled by RLA-I (control and isolated) rats. *p < 0.05, **p < 0.01, one-way ANOVA following significant overall ANOVA.

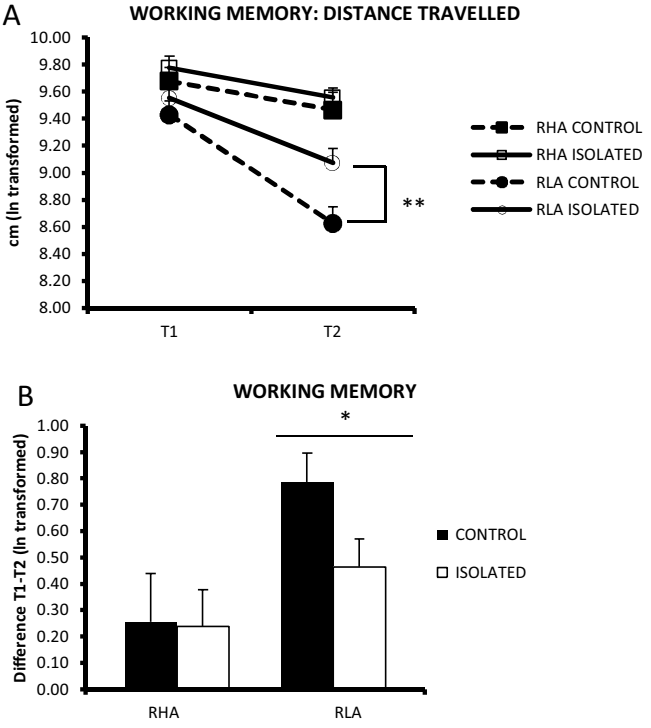


Fig. 4. (A) Mean ± S.E.M. of the distance (cm) travelled by the rats in the first (T1) and second (T2) trials, averaged for the four days. B) Mean ± S.E.M. of the differences (T1-T2), between the first (T1) and the second trials (T2) of the four days of working memory task. *p < 0.05;**p < 0.01, one-way ANOVA (A)/ANCOVA (B) following significant overall ANOVA (A)/ANCOVA (B).

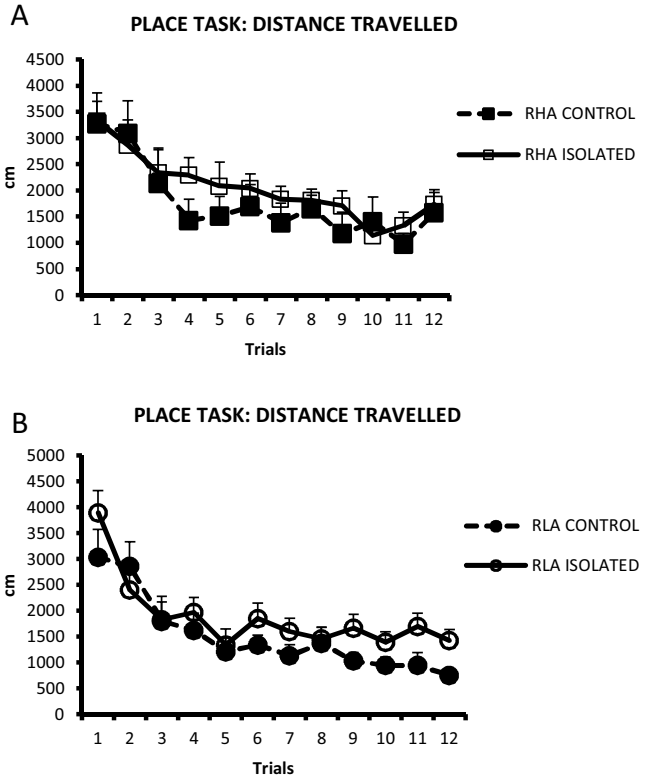


Fig. 5. Mean ± S.E.M. of the distance (cm) travelled by RHA-I (A) and RLA-I (B) (control and isolated) rats in the 12 trials of the place learning task (3 trials/day, 4 days).

(approximately 19% on average) longer distances than group-housed rats, while RLA-I rats were unaffected. Regarding spatial learning/memory in the MWM, isolation rearing produced a deficit of long-term spatial reference memory (i.e. transfer test) only in RHA-I rats. Effects of IR on RLA-I rats were restricted to an impairment in spatial working memory. Finally, in the cued learning task

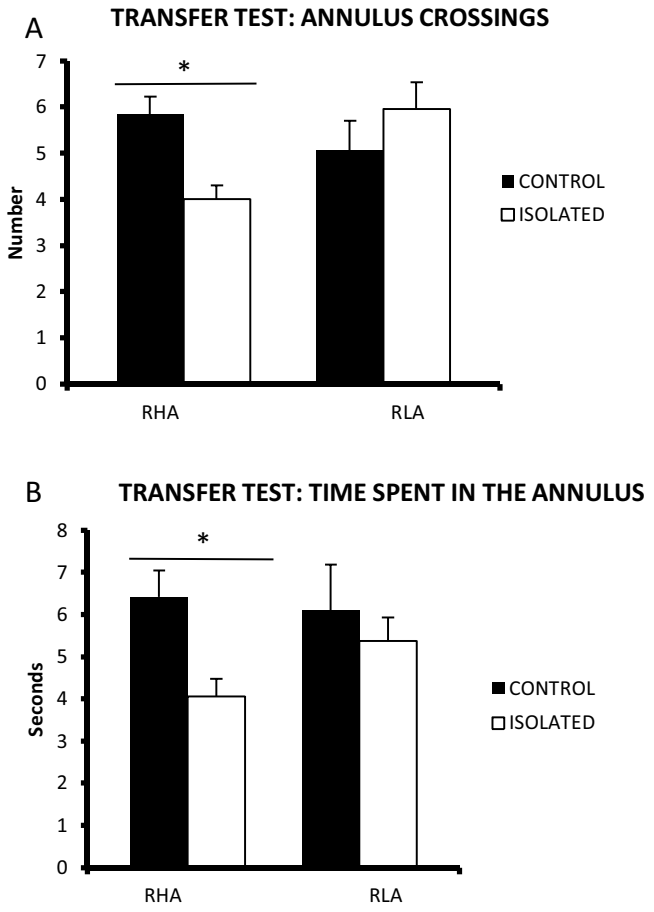


Fig. 6. (A) Mean ± S.E.M. of annulus crossings (i.e. the number of times the rats swam across the annulus where the platform was located during the place learning task; 60-s trial) and (B) mean ± S.E.M. of time spent in the annulus from each group. * $P \leq 0.002$, one-way ANOVA following significant overall ANOVA.

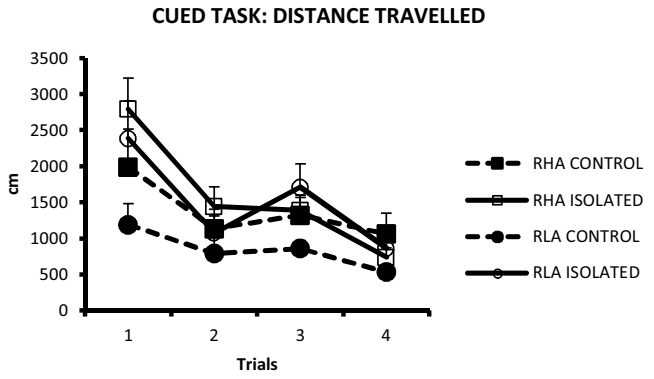


Fig. 7. (A) Mean ± S.E.M. distance (cm) travelled by the rats of each strain (RHA-I and RLA-I) and treatment (control and isolated) for each of the four trials of the cued task.

there wasn't any significant strain or treatment effect, indicating that the effects in the learning/memory tasks were not due to any gross physical, sensory or motivational differences among groups. Besides these effects of isolation rearing, the observed between-strain differences (i.e. "strain" main effects) are in line with what is known from the Roman rat strains, i.e. RLA-I rats present better PPI and working memory performance than RHA-Is, while the latter are less anxious and display higher levels of novelty-induced horizontal activity than the former (e.g Refs. [16,13,39,17,12])

A large number of studies have shown that isolation rearing leads to PPI impairments in rats and mice (e.g. Refs. [40–49], for review see Refs. [1,2,50]). It has been suggested that isolation-induced disruption of PPI could be due to changes in dopaminergic transmission, as it can be reversed by treatment with non-sedative doses of the selective, D2 antagonist raclopride [45]. Further work has shown than also atypical antipsychotic drugs reverse IR-induced PPI disruption, thus implicating serotonin neurotransmission in that deficit (for review see Ref. [1]). However, the robustness of the IR-PPI phenomenon in rats depends on the strain to some extent, with some strains even showing an absence of –or resistance to- IR effects (for review see Refs. [1,50]).

The possibility that IR-induced PPI disruption needs a critical time window to appear has been evaluated and discussed by several authors (e.g. Refs. [43,44,51]). Liu et al. [51]. suggest that this factor could explain the variability in the results of different experiments. For instance, Varty et al. [48] used different social isolation periods to see how many weeks were needed to induce the PPI deficit, and they found that 8 weeks were sufficient to induce it whereas shorter time windows were insufficient. On the other hand, Liu et al. [51] found that 2 weeks of social isolation were enough to observe disruptive effects of IR on PPI. Another study that shows the variability in the onset of IR-PPI disruption among strains is Bakshi and Geyer [52]. They found that the PPI deficit in Sprague-Dawley rats was present after 4 weeks of isolation, whereas Lister rats needed 7 weeks of IR in order to exhibit the PPI impairment. Compared to these studies, and in order to maximize the expected IR effects, we have used a longer isolation period, i.e. 14 weeks at the beginning of PPI testing, 16 and 18 weeks at the time of anxiety and activity testing, respectively.

Another possible confounding factor in IR-PPI studies, which has been demonstrated to influence results, is testing order when multiple tests (besides PPI) are carried out. The study by Domeney and Feldon [43] is important in this regard, as the authors demonstrated that IR-induced PPI disruption in rats was only observed when PPI was the first test of a test battery (they also tested IR rats in activity cages and in the open field), but not when it was preceded by the activity tests. Accordingly, in the present study we have first tested animals for PPI. We have observed a significant PPI deficit in the RHA-I rat strain, particularly evident at the lowest prepulse intensity, which suggests that the IR-induced sensorimotor gating deficits in this rat strain become evident at relatively low prepulse intensities (i.e. those close to the background noise intensity and to the lowest prepulse threshold leading to PPI). In any case, these results are consistent with previous studies suggesting that RHA-I rats may be a valid model of some schizophrenia-related endophenotypes, such as PPI [16,21,17]. Interestingly, RLA-I rats seem to be resistant to that IR-induced PPI disruption.

Lukkes et al. [53] reviewed the effects of isolation rearing (and social deprivation in adulthood) on anxiety, fear and stress reactivity in rats. These authors conclude that the most general findings appear to be that IR leads to increases in anxiety and fear, whereas IR effects on hypothalamus-pituitary-adrenal (HPA) stress responses are inconsistent. Some of the inconsistencies across works may be due to the use of re-socialization (after several weeks of isolation rearing) in some studies (for review see Ref. [53]). The same seems to apply to IR-induced hyperactivity, a phenomenon that appears to be robust (e.g. Ref. [52,54,44]; reviewed by [1,2]) but that can also be normalized by re-socialization (e.g. Ref. [51]). This is a reason why in the present study rats were not re-socialized. Our results show that IR clearly increased anxious behavior (i.e. reduction of "time spent into open sections", "entries in open sections" and "head-dipping" in the elevated zero-maze) and induced hyperactivity (in the activity cages) in RHA-I rats, while leaving RLA-Is unaffected. Interestingly, the anxiety scores of isolated RHA-I rats reached the same levels as those from RLA-I rats, although it

cannot be completely ruled out that the absence of IR effects on anxiety in RLA-Is could be due a floor effect (as control rats from this strain show very low scores of entries into or time spent in the open sections of the elevated zero-maze; see Fig. 2). Moreover, the fact that IR-induced hyperactivity is observed only in RHA-I rats adds further support to the validity of this strain to model some schizophrenia-relevant features.

Regarding the results found in the different spatial learning/memory tasks in the MWM, IR impaired reference memory (i.e. transfer test) in the RHA-I strain and disrupted working memory in RLA-I rats, while place and cued learning were unaffected. It cannot be ruled out that the absence of IR effects on working memory in the RHA-I strain is due to a floor effect, as they present poor performance in this task (see also Ref. [17]). Results from previous studies of IR effects on these spatial tasks in rats have also been heterogeneous. Thus, for instance, Quan et al. [55] showed that rats reared in isolation showed an impairment in the transfer test, i.e. reference memory. However, both Quan et al. [55] and Schrijver et al. [56] found no differences between IR and group-housed rats in a place learning task in the MWM, and Wongwitdecha and Marsden [57] even reported that IR improved place learning performance. The authors suggested that isolation could be affecting the persistence of spatial memory while leaving place learning unaffected [55], as it appears to be the case in the present study, in which we only found a non-significant tendency of isolated rats to travel longer distances compared to group-housed rats. Also in line with Quan et al. [55] we have observed an IR-induced disruption of reference memory in the transfer test, but only in RHA-I rats. The impairing effects of isolation rearing on reversal learning –in which animals must show cognitive flexibility and learn about changing "rules"- appear to be much more consistent than those on place (reference) learning, in which the platform remains in a fixed position across trials and training days (for review see Ref. [1]). This, and the review of neurobiological effects of IR, has led to the proposal that IR may primarily affect the cortico-striatal pathways involved in reversal learning, rather than the cortico-hippocampal neuronal pathways implicated in spatial place learning and memory [1]. Conversely, IR impaired spatial working memory in the RLA-I strain, which typically shows better performance in this task than the RHA-I strain (see also Refs. [22,17]). Likewise, in the only study, to our knowledge, that has evaluated working memory in the MWM in isolated rats [58], an IR-induced impairment was observed. Similarly, Sandstrom and Hart [59] found that rats reared in isolation for 6 h/day during postnatal days 15–21 made more working memory errors (while the effect of IR on reference memory was not significant) than non-isolated rats when tested in the radial-arm maze during adulthood. As the working memory tasks require learning different rules in different days (in contrast to the "single rule" learning that characterizes reference –place- learning) it would be interesting to explore whether cortico-striatal pathways are preferentially involved, as it has been proposed for reversal learning tasks (see Ref. [1]).

Alternatively, it has been shown that isolation rearing leads to neurochemical/functional effects (e.g. muscarinic receptors, serotonin transmission, noradrenergic receptors, and others) affecting hippocampal function (e.g Ref. [60,61]; for review see Ref. [53]), and structural/morphological changes in the hippocampus (i.e. decreased spine density and fewer synapses) have also been found in isolation-reared rats [62–65], resembling the changes observed in schizophrenic patients [66,67]. As reported by Meyza et al. [34] and Garcia-Falgueras et al. [35], RHA-I rats show a decreased hippocampal activity and neuronal density, clearly suggesting that RHA-I rats present a less functional and perhaps more vulnerable hippocampus (recent unpublished results show reduced hippocampal volumes in RHA-I vs RLA-I rats using structural MRI; Rio-Alamos et al. unpublished). The hippocampus has been implicated in the majority of the processes measured by the tests we have conducted, i.e. PPI [68,69], anxiety [70,53,12] and spatial learning/memory tasks [71–73]. Thus, those IR-induced hippocampal changes, together with the apparently more vulnerable (or less functional) hippocampus of the RHA-I strain, could (at least partly) explain why isolated RHA-I rats exhibit almost all of the features of the so-called "isolation syndrome" [2], i.e. PPI deficits (see Fig. 1A), increased anxiety (see Fig. 2A–E), hyperactivity (see Fig. 3A–B), cognitive/learning/memory impairments (see Fig. 6A–B)

The isolation-rearing syndrome in rats is considered a model of early adversity-induced schizophrenia-like symptoms/features. Compared with RLA-I rats, IR has a more powerful impact in the RHA-I strain, as they exhibit isolation-induced PPI deficits, increased anxiety, hyperactivity and long-term reference memory deficits, while isolated RLA-I rats only exhibit deficits in the spatial working memory task. This profile, adds to the recently reported PPI and latent inhibition deficits of RHA-I rats (compared with both RLA-I rats as well as with a reference control such as the genetically heterogeneous NIH-HS rats [17,74]), and to the finding that PPI deficit of RHA-I rats can be reversed by the antipsychotic haloperidol (while the drug does not affect PPI in RLA-I rats; Oliveras et al. unpublished data). Collectively, the above strain-related profiles, jointly with the aforementioned between-strain pharmacological/neurochemical and structural differences, are consistent with the contention that the RHA-I rat strain may be a valid genetically-based vulnerability model to study schizophrenia-relevant features (see also Refs. [16,17]).

Acknowledgements

This work was supported by grants PSI2013-41872-P (MINECO), 2014SGR-1587 (DGR), "ICREA-Academia 2013" (to AF-T). I.O is recipient of a PhD FI fellowship (DGR 2014). A.S-G is recipient of a PhD FPI fellowship. MA.P gratefully acknowledges Sardinia Regional Government for the financial support of her PhD scholarship (P.O.R. Sardegna F.S.E. Operational Programme of the Autonomous Region of Sardinia, European Social Fund 2007–2013 – Axis IV Human Resources, Objective l.3, Line of Activity l.3.1.)

References

[1] K.C.F. Fone, M.V. Porkess, Behavioural and neurochemical effects of post-weaning social isolation in rodents: relevance to developmental neuropsychiatric disorders, Neurosci. Biobehav. Rev. 32 (2008) 1087–1102, http://dx.doi.org/10.1016/j.neubiorev.2008.03.003.

[2] C.A. Jones, D.J.G. Watson, K.C.F. Fone, Animal models of schizophrenia, Br. J. Pharmacol. 164 (2011) 1162–1194, http://dx.doi.org/10.1111/j.1476-5381.2011.01386.x.

[3] L. Valzelli, The isolation syndrome in mice, Psychopharmacologia 31 (1973) 305–320, http://dx.doi.org/10.1007/bf00421275.

[4] D.F. Einon, M.J. Morgan, A critical period for social isolation in the rat, Dev. Psychobiol. 10 (1977) 123–132, http://dx.doi.org/10.1002/dev.420100205.

[5] C.A. Heidbreder, I.C. Weiss, A.M. Domeney, C. Pryce, J. Homberg, G. Hedou, et al., Behavioral, neurochemical and endocrinological characterization of the early social isolation syndrome, Neuroscience 100 (2000) 749–768, http://dx.doi.org/10.1016/S0306-4522(00)00336-5.

[6] I.C. Weiss, C.R. Pryce, A.L. Jongen-Rêlo, N.I. Nanz-Bahr, J. Feldon, Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat, Behav. Brain Res. 152 (2004) 279–295, http://dx.doi.org/10.1016/j.bbr.2003.10.015.

[7] C.A. Marsden, M.V. King, K.C.F. Fone, Influence of social isolation in the rat on serotonergic function and memory?relevance to models of schizophrenia and the role of 5-HT_6 receptors, Neuropharmacology 61 (2011) 400–407, http://dx.doi.org/10.1016/j.neuropharm.2011.03.003.

[8] P. Driscoll, A. Fernández-Teruel, M.G. Corda, O. Giorgi, Steimer, T, Some guidelines for defining personality differences in rats, in: K. Yong-Kyu (Ed.), Handbook of Behavior Genetics, Springer, New York, NY, 2009, pp. 281–300.

[9] R.M. Escorihuela, A. Fernández-Teruel, L. Gil, R. Aguilar, A. Tobeña, P. Driscoll, Inbred Roman high- and low-avoidance Rats: differences in anxiety, novelty-seeking, and shuttlebox behaviors, Physiol. Behav. 67 (1999) 19–26, http://dx.doi.org/10.1016/S0031-9384(99)00064-5.

[10] R. López-Aumatell, E. Vicens-Costa, M. Guitart-Masip, E. Martínez-Membrives, W. Valdar, M. Johannesson, et al., Unlearned anxiety predicts learned fear: a comparison among heterogeneous rats and the Roman rat strains, Behav. Brain Res. 202 (2009) 92–101, http://dx.doi.org/10.1016/j.bbr.2009.03.024.
[11] R. López-Aumatell, G. Blázquez, L. Gil, R. Aguilar, T. Cañete, L. Giménez-Llort, et al., The Roman high- and low-avoidance rat strains differ in fear-potentiated startle and classical aversive conditioning, Psicothema 21 (2009) 27–32.
[12] C. Río-Álamos, I. Oliveras, T. Cañete, G. Blázquez, E. Martínez-Membrives, A. Tobeña, A. Fernández-Teruel, Neonatal handling decreases unconditioned anxiety, conditioned fear, and improves two-way avoidance acquisition: a study with the inbred Roman high (RHA-I)- and low-avoidance (RLA-I) rats of both sexes, Front. Behav. Neurosci. 9 (2015) 174, http://dx.doi.org/10.3389/fnbeh.2015.00174.
[13] S. Díaz-Morán, M. Palència, C. Mont-Cardona, T. Cañete, G. Blázquez, E. Martínez-Membrives, et al., Coping style and stress hormone responses in genetically heterogeneous rats: comparison with the Roman rat strains, Behav. Brain Res. 228 (2012) 203–210, http://dx.doi.org/10.1016/j.bbr.2011.12.002.
[14] T. Steimer, P. Driscoll, Divergent stress responses and coping styles in psychogenetically selected Roman high- (RHA) and low-(RLA) avoidance rats: behavioural, neuroendocrine and developmental aspects, Stress 6 (2003) 87–100, http://dx.doi.org/10.1080/1025389031000111320.
[15] J. Carrasco, C. Márquez, R. Nadal, A. Tobeña, A. Fernández-Teruel, A. Armario, Characterization of central and peripheral components of the hypothalamus-pituitary-adrenal axis in the inbred Roman rat strains, Psychoneuroendocrinology 33 (2008) 437–445, http://dx.doi.org/10.1016/j.psyneuen.2008.01.001.
[16] C. Del Río, I. Oliveras, T. Cañete, G. Blázquez, A. Tobeña, A. Fernández-Teruel, Genetic rat models of schizophrenia-relevant symptoms, World J. Neurosci. 4 (2014) 261–278, http://dx.doi.org/10.4236/wjns.2014.43030.
[17] I. Oliveras, C. Río-Álamos, T. Cañete, G. Blázquez, E. Martínez-Membrives, O. Giorgi, et al., Prepulse inhibition predicts spatial working memory performance in the inbred Roman high- and low-avoidance rats and in genetically heterogeneous NIH-HS rats: relevance for studying pre-attentive and cognitive anomalies in schizophrenia, Front. Behav. Neurosci. 9 (2015) 213, http://dx.doi.org/10.3389/fnbeh.2015.00213.
[18] H. Zeier, K. Baettig, P. Driscoll, Acquisition of DRL-20 behavior in male and female, Roman high- and low-avoidance rats, Physiol. Behav. 20 (1978) 791–793, http://dx.doi.org/10.1016/0031-9384(78)90307-4.
[19] M. Moreno, D. Cardona, M.J. Gómez, F. Sánchez-Santed, A. Tobeña, A. Fernández-Teruel, et al., Impulsivity characterization in the Roman high- and low-avoidance rat strains: behavioral and neurochemical differences, Neuropsychopharmacology 35 (2010) 1198–1208, http://dx.doi.org/10.1038/npp.2009.224.
[20] A.B. Klein, L. Ultved, D. Adamsen, M.A. Santini, A. Tobeña, A. Fernandez-Teruel, et al., 5-HT(2A) and mGlu2 receptor binding levels are related to differences in impulsive behavior in the Roman low-(RLA) and high-(RHA) avoidance rat strains, Neuroscience 263 (2014) 36–45, http://dx.doi.org/10.1016/j.neuroscience.2013.12.063.
[21] A. Fernández-Teruel, G. Blázquez, M. Pérez, R. Aguilar, T. Canete, M. Guitart, et al., Latent inhibition threshold in Roman high-avoidance rats: a psychogenetic model of abnormalities in attentional filter? Actas Españolas Psiquiatria 34 (2006) 257–263.
[22] R. Aguilar, R.M. Escorihuela, L. Gil, A. Tobeña, A. Fernández-Teruel, Differences between two psychogenetically selected lines of rats in a swimming pool matching-to-place task: long-term effects of infantile stimulation, Behav. Genet. 32 (2002) 127–134, http://dx.doi.org/10.1023/A:1015253807488.
[23] P. Driscoll, P. Ferré, A. Fernández-Teruel, M. Levi de Stein, C. Wolfman, J. Medina, et al., Effects of prenatal diazepam on two-way avoidance behavior, swimming navigation and brain levels of benzodiazepine-like molecules in male Roman high- and low-avoidance rats, Psychopharmacology (Berl.) 122 (1995) 51–57, http://dx.doi.org/10.1007/bf02246441.
[24] R.M. Escorihuela, A. Tobena, A. Fernández-Teruel, Environmental enrichment and postnatal handling prevent spatial learning deficits in aged hypoemotional (Roman high-avoidance) and hyperemotional (Roman low-avoidance) rats, Learn. Mem. 2 (1995) 40–48, http://dx.doi.org/10.1101/lm.2.1.40.
[25] E. Martínez-Membrives, R. López-Aumatell, G. Blázquez, T. Cañete, A. Tobeña, A. Fernández-Teruel, Spatial learning in the genetically heterogeneous NIH-HS rat stock and RLA-I/RHA-I rats: revisiting the relationship with unconditioned and conditioned anxiety, Physiol. Behav. 144 (2015) 15–25, http://dx.doi.org/10.1016/j.physbeh.2015.03.003.
[26] M.G. Corda, G. Piras, D. Lecca, A. Fernández-Teruel, P. Driscoll, O. Giorgi, The psychogenetically selected Roman rat lines differ in the susceptibility to develop amphetamine sensitization, Behav. Brain Res. 157 (2005) 147–156, http://dx.doi.org/10.1016/j.bbr.2004.06.016.
[27] O. Giorgi, D. Lecca, G. Piras, P. Driscoll, M.G. Corda, Dissociation between mesocortical dopamine release and fear-related behaviours in two psychogenetically selected lines of rats that differ in coping strategies to aversive conditions, Eur. J. Neurosci. 17 (2003) 2716–2726, http://dx.doi.org/10.1046/j.1460-9568.2003.02689.x.
[28] M. Guitart-Masip, B. Johansson, T. Cañete, A. Fernández-Teruel, A. Tobeña, L. Terenius, L. Giménez-Llort, Regional adaptations in PSD-95, NGFI-A and secretogranin gene transcripts related to vulnerability to behavioral sensitization to amphetamine in the Roman rat strains, Neuroscience 151 (2008) 195–208, http://dx.doi.org/10.1016/j.neuroscience.2007.09.072.
[29] O. Giorgi, G. Piras, M.G. Corda, The psychogenetically selected Roman high- and low-avoidance rat lines: a model to study the individual vulnerability to drug addiction, Neurosci. Biobehav. Rev. 31 (2007) 148–163, http://dx.doi.org/10.1016/j.neubiorev.2006.07.008.
[30] L. Giménez-Llort, T. Cañete, M. Guitart-Masip, A. Fernández-Teruel, A. Tobeña, Two distinctive apomorphine-induced phenotypes in the Roman high- and low-avoidance rats, Physiol. Behav. 86 (2005) 458–466, http://dx.doi.org/10.1016/j.physbeh.2005.07.021.
[31] F. Sanna, M.G. Corda, M.R. Melis, M.A. Piludu, O. Giorgi, A. Argiolas, Male Roman high and low avoidance rats show different patterns of copulatory behaviour: comparison with Sprague Dawley rats, Physiol. Behav. 127 (2014) 27–36, http://dx.doi.org/10.1016/j.physbeh.2014.01.002.
[32] F. Sanna, M.A. Piludu, M.G. Corda, A. Argiolas, O. Giorgi, M.R. Melis, Dopamine is involved in the different patterns of copulatory behaviour of Roman high and low avoidance rats: studies with apomorphine and haloperidol, Pharmacol. Biochem. Behav. 124 (2014) 211–219, http://dx.doi.org/10.1016/j.pbb.2014.06.012.
[33] J. Sallés, M. López de Jesús, O. Goñi, A. Fernández-Teruel, P. Driscoll, A. Tobeña, Transmembrane signaling through phospholipase C in cortical and hippocampal membranes of psychogenetically selected rat lines, Psychopharmacology (Berl.) 154 (2001) 115–125, http://dx.doi.org/10.1007/s002130000621.
[34] K.Z. Meyza, P.M. Boguszewski, E. Nikolaev, J. Zagrodzka, Diverse sensitivity of RHA/Verh and RLA/Verh rats to emotional and spatial aspects of a novel environment as a result of a distinct pattern of neuronal activation in the fear/anxiety circuit, Behav. Geneti. 39 (2009) 48–61, http://dx.doi.org/10.1007/s10519-008-9234-z.
[35] A. Garcia-Falgueras, M.M. Castillo-Ruiz, T. Put, A. Tobeña, A. Fernández-Teruel, Differential hippocampal neuron density between inbred Roman high- (low anxious) and low-avoidance (high anxious) rats, Neurosci. Lett. 522 (2012) 41–46, http://dx.doi.org/10.1016/j.neulet.2012.06.011.
[36] A. Ellaithy, J. Younkin, J. González-Maeso, D.E. Logothetis, Positive allosteric modulators of metabotropic glutamate 2 receptors in schizophrenia treatment, Trends Neurosci. 38 (2015) 506–516, http://dx.doi.org/10.1016/j.tins.2015.06.002.
[37] J.K. Shepherd, S.S. Grewal, A. Fletcher, D.J. Bill, C.T. Dourish, Behavioural and pharmacological characterisation of the elevated zero-maze as an animal model of anxiety, Psychopharmacology (Berl.) 116 (1994) 56–64, http://dx.doi.org/10.1007/BF02244871.
[38] R. López-Aumatell, M. Guitart-Masip, E. Vicens-Costa, L. Giménez-Llort, W. Valdar, M. Johannesson, et al., Fearfulness in a large N/Nih genetically heterogeneous rat stock: differential profiles of timidity and defensive flight in males and females, Behav. Brain Res. 188 (2008) 41–55, http://dx.doi.org/10.1016/j.bbr.2007.10.015.
[39] C. Estanislau, S. Díaz-Morán, T. Cañete, G. Blázquez, A. Tobeña, A. Fernández-Teruel, Context-dependent differences in grooming behavior among the NIH heterogeneous stock and the Roman high- and low-avoidance rats, Neurosci. Res. 77 (2013) 187–201, http://dx.doi.org/10.1016/j.neures.2013.09.012.
[40] Y. Ago, R. Araki, K. Yano, T. Kawasaki, S. Chaki, A. Nakazato, et al., The selective metabotropic glutamate 2/3 receptor agonist MGS0028 reverses isolation rearing-induced abnormal behaviors in mice, J. Pharmacol. Sci. 118 (2012) 295–298, http://dx.doi.org/10.1254/jphs.11200SC.
[41] J. Cilia, C. Reavill, J. Hagan, D. Jones, Long-term evaluation of isolation-rearing induced prepulse inhibition deficits in rats, Psychopharmacology 156 (2001) 327–337, http://dx.doi.org/10.1007/s002130100786.
[42] V.P. Bakshi, N.R. Swerdlow, D.L. Braff, M.A. Geyer, Reversal of isolation rearing-induced deficits in prepulse inhibition by Seroquel and olanzapine, Biol. Psychiatry 43 (1998) 436–445, http://dx.doi.org/10.1016/S0006-3223(97)00246-1.
[43] A. Domeney, J. Feldon, The disruption of prepulse inhibition by social isolation in the wistar rat: how robust is the effect? Pharmacol. Biochem. Behav. 59 (1998) 883–890, http://dx.doi.org/10.1016/S0091-3057(97)00534-0.
[44] K. Fabricius, L. Helboe, A. Fink-Jensen, G. Wörtwein, B. Steiniger-Brach, Pharmacological characterization of social isolation-induced hyperactivity, Psychopharmacology 215 (2011) 257–266, http://dx.doi.org/10.1007/s00213-010-2128-9.
[45] M.A. Geyer, L.S. Wilkinson, T. Humby, Isolation rearing of rats produces a deficit in prepulse inhibition of acoustic startle similar to that in schizophrenia, Biol. Psychiatry 34 (1993) 361–372, http://dx.doi.org/10.1016/0006-3223(93)90180-l.
[46] M.V. Porkess, The Impact of Social Isolation on Rat Behaviour. Dissertation, (March), 2008, Retrieved from http://etheses.nottingham.ac.uk/507/.
[47] S.B. Powell, N.R. Swerdlow, L.K. Pitcher, M.A. Geyer, Isolation rearing-induced deficits in prepulse inhibition and locomotor habituation are not potentiated by water deprivation, Physiol. Behav. 77 (2002) 55–64, http://dx.doi.org/10.1016/S0031-9384(02)00817-X.
[48] G.B. Varty, D.L. Braff, M.A. Geyer, Is there a critical developmental window for isolation rearing-induced changes in prepulse inhibition of the acoustic startle response? Behav. Brain Res. 100 (1999) 177–183, http://dx.doi.org/10.1016/S0166-4328(98)00129-6.
[49] L.S. Wilkinson, S.S. Killcross, T. Humby, F.S. Hall, M.A. Geyer, T.W. Robbins, Social isolation in the rat produces developmentally specific deficits in prepulse inhibition of the acoustic startle response without disrupting latent

inhibition, Neuropsychopharmacology 10 (1994) 61–72, http://dx.doi.org/10.1038/npp.1994.8.
[50] I.C. Weiss, J. Feldon, Environmental animal models for sensorimotor gating deficiencies in schizophrenia: a review, Psychopharmacology 156 (2001) 305–326, http://dx.doi.org/10.1007/s002130100800.
[51] Y.P. Liu, Y.C. Kao, C.S. Tung, Critical period exists in the effects of isolation rearing on sensorimotor gating function but not locomotor activity in rat, Progress Neuro-Psychopharmacol. Biol. Psychiatry 35 (2011) 1068–1073, http://dx.doi.org/10.1016/j.pnpbp.2011.03.002.
[52] V.P. Bakshi, M.A. Geyer, Ontogeny of isolation rearing-induced deficits in sensorimotor gating in rats, Physiol. Behav. 67 (1999) 385–392, http://dx.doi.org/10.1016/S0031-9384(99)00082-7.
[53] J.L. Lukkes, M.J. Watt, C.A. Lowry, G.L. Forster, Consequences of post-weaning social isolation on anxiety behavior and related neural circuits in rodents, Front. Behav. Neurosci. 3 (2009) 18, http://dx.doi.org/10.3389/neuro.08.018.2009.
[54] F.S. Hall, S. Huang, G.W. Fong, A. Pert, M. Linnoila, Effects of isolation-rearing on locomotion, anxiety and responses to ethanol in Fawn Hooded and Wistar rats, Psychopharmacology (Berl.) 139 (1998) 203–209, http://dx.doi.org/10.1007/s002130050705.
[55] M.N. Quan, Y.T. Tian, K.H. Xu, T. Zhang, Z. Yang, Post weaning social isolation influences spatial cognition, prefrontal cortical synaptic plasticity and hippocampal potassium ion channels in Wistar rats, Neuroscience 169 (2010) 214–222, http://dx.doi.org/10.1016/j.neuroscience.2010.04.048.
[56] N.C. Schrijver, N.I. Bahr, I.C. Weiss, H. Würbel, Dissociable effects of isolation rearing and environmental enrichment on exploration, spatial learning and HPA activity in adult rats, Pharmacol. Biochem. Behav. 73 (2002) 209–224, http://dx.doi.org/10.1016/S0091-3057(02)00790-6.
[57] N. Wongwitdecha, C.A. Marsden, Social isolation increases aggressive behaviour and alters the effects of diazepam in the rat social interaction test, Behav. Brain Res. 75 (1996) 27–32, http://dx.doi.org/10.1016/0166-4328(96)00181-7.
[58] H. Famitafreshi, M. Karimian, H. Fanaei, F. Attari, S. Fatima, Social isolation is associated with reduced neurogenesis, impaired spatial working memory performance, and altered anxiety levels in male rats, Open Access Anim. Physiol. 7 (2015) 87–95, http://dx.doi.org/10.2147/OAAP.S84327.
[59] N.J. Sandstrom, S.R. Hart, Isolation stress during the third postnatal week alters radial arm maze performance and corticosterone levels in adulthood, Behav. Brain Res. 156 (2005) 289–296, http://dx.doi.org/10.1016/j.bbr.2004.05.033.
[60] S. Muchimapura, R. Mason, C.A. Marsden, Effect of isolation rearing on pre- and post-synaptic serotonergic function in the rat dorsal hippocampus, Synapse 47 (2003) 209–217, http://dx.doi.org/10.1002/syn.10167.
[61] A.J. Fulford, C.A. Marsden, Social isolation in the rat enhances alpha 2-autoreceptor function in the hippocampus in vivo, Neuroscience 77 (1997) 57–64, http://dx.doi.org/10.1016/S0306-4522(96)00499-X.
[62] M. Bianchi, K.C.F. Fone, N. Azmi, C.A. Heidbreder, J.J. Hagan, C.A. Marsden, Isolation rearing induces recognition memory deficits accompanied by cytoskeletal alterations in rat hippocampus, Eur. J. Neurosci. 24 (2006) 2894–2902, http://dx.doi.org/10.1111/j.1460-9568.2006.05170.x.
[63] K. Day-Wilson, D.N.C. Jones, E. Southam, J. Cilia, S. Totterdell, Medial prefrontal cortex volume loss in rats with isolation rearing-induced deficits in prepulse inhibition of acoustic startle, Neuroscience 141 (2006) 1113–1121, http://dx.doi.org/10.1016/j.neuroscience.2006.04.048.
[64] L. Lu, G.B. Bao, H. Chen, P. Xia, X.L. Fan, J.S. Zhang, et al., Modification of hippocampal neurogenesis and neuroplasticity by social environments, Exp. Neurol. 183 (2003) 600–609, http://dx.doi.org/10.1016/S0014-4886(03)00248-6.
[65] G.B. Varty, C.A. Marsden, G.A. Higgins, Reduced synaptophysin immunoreactivity in the dentate gyrus of prepulse inhibition-impaired isolation-reared rats, Brain Res. 824 (1999) 197–203, http://dx.doi.org/10.1016/S0006-8993(99)01173-7.
[66] P.J. Harrison, The hippocampus in schizophrenia: a review of the neuropathological evidence and its pathophysiological implications, Psychopharmacology (Berl.) 174 (151) (2004) 162, http://dx.doi.org/10.1007/s00213-003-1761-y.
[67] S. Heckers, C. Konradi, Hippocampal neurons in schizophrenia, J. Neural Transm. 109 (2002) 891–905, http://dx.doi.org/10.1007/s007020200073.
[68] S. Kohl, K. Heekeren, J. Klosterkötter, Prepulse inhibition in psychiatric disorders-apart from schizophrenia, J. Psychiatr. Res. 47 (2013) 445–452, http://dx.doi.org/10.1016/j.jpsychires.2012.11.018.
[69] N. Swerdlow, M. Geyer, D. Braff, Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges, Psychopharmacology 156 (2001) 194–215, http://dx.doi.org/10.1007/s002130100799.
[70] J.A. Gray, N. McNaughton, The Neuropsychology of Anxiety: An Enquiry into the Functions of the Septo-Hippocampal System, 2 ed., Oxford University Press, Oxford, 2000 (Oxford Psychology Series No. 33).
[71] R.G.M. Morris, E. Anderson, G.S. Lynch, M. Baudry, Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartat receptor antagonist, AP5, Nature 319 (1986) 774–776, http://dx.doi.org/10.1038/319774a0.
[72] R.G.M. Morris, J.J. Hagan, J.N.P. Rawlins, Allocentric spatial learning by hippocampectomized rats: a further test of the spatial mapping and working memory theories of hippocampal function, Q. J. Exp. Psychol. 38B (1986) 365–395, http://dx.doi.org/10.1080/14640748608402242.
[73] C.G. Wible, Hippocampal physiology, structure and function and the neuroscience of schizophrenia: a unified account of declarative memory deficits, working memory deficits and schizophrenic symptoms, Behav. Sci. 3 (2013) 298–315, http://dx.doi.org/10.3390/bs3020298.
[74] A. Esnal, A. Sánchez-González, C. Río-Álamos, I. Oliveras, T. Cañete, G. Blázquez, A. Tobeña, A. Fernández-Teruel, Prepulse inhibition and latent inhibition deficits in Roman high-avoidance vs. Roman low-avoidance rats: modeling schizophrenia-related features, Physiol. Behav. 163 (2016) 267–273, http://dx.doi.org/10.1016/j.physbeh.2016.05.020.

11. ESTUDI 3: doi:10.1007/s00213-017-4534-8

Differential effects of antipsychotic and propsychotic drugs on prepulse inhibition and locomotor activity in Roman high- (RHA) and low-avoidance (RLA) rats

(Vegeu “Copyright Springer” a l’annex 2)

Differential effects of antipsychotic and propsychotic drugs on prepulse inhibition and locomotor activity in Roman High- (RHA) and Low-Avoidance (RLA) rats

Ignasi Oliveras*, Ana Sánchez-González, Daniel Sampedro-Viana, Maria Antonietta Piludu[1], Cristóbal Río-Alamos, Osvaldo Giorgi[1], Maria G. Corda[1], Susana Aznar[2], Javier González-Maeso[3], Cristina Gerbolés, Gloria Blázquez, Toni Cañete, Adolf Tobeña, Alberto Fernández-Teruel*

Psychopharmacology (2017) 234:957–975 **DOI** 10.1007/s00213-017-4534-8

Department of Psychiatry & Forensic Medicine, Institute of Neurosciences, Universidad Autónoma de Barcelona, 08193 Bellaterra, Barcelona, Spain.

[1]Department of Life and Environmental Sciences, Section of Pharmaceutical, Pharmacological and Nutraceutical Sciences, University of Cagliari, 09124 Cagliari, Italy.

[2]Research Laboratory for Stereology and Neuroscience, Bispebjerg and Frederiksberg Hospitals, Copenhagen, Denmark

[3]Department of Physiology and Biophysics, Virginia Commonwealth University School of Medicine, Richmond, Virginia 23298

*Corresponding authors: Alberto Fernández-Teruel & Ignasi Oliveras

ABSTRACT

Rationale

Animal models with predictive and construct validity are necessary for developing novel and efficient therapeutics for psychiatric disorders.

Objectives

We have carried out a pharmacological characterization of the Roman high- (RHA-I) and low-avoidance (RLA-I) rat strains with different acutely administered propsychotic (DOI, MK-801) and antipsychotic drugs (haloperidol, clozapine), as well as apomorphine, on prepulse inhibition (PPI) of startle and locomotor activity (activity cages).

Results

RHA-I rats display a consistent deficit of PPI compared with RLA-I rats. The typical antipsychotic haloperidol (dopamine D-2 receptor antagonist) reversed the PPI deficit characteristic of RHA-I rats (in particular at 65 and 70 dB prepulse intensities) and reduced locomotion in both strains. The atypical antipsychotic clozapine (serotonin/dopamine receptor antagonist) did not affect PPI in either strain, but decreased locomotion in a dose-dependent manner in both rat strains. The mixed dopamine D1/D2 agonist, apomorphine, at the dose of 0.05 mg/kg, decreased PPI in RHA-I, but not RLA-I rats. The hallucinogen drug DOI (5-HT2A agonist; 0.1 - 1.0 mg/kg) disrupted PPI in RLA-I rats in a dose-dependent manner at the 70 dB prepulse intensity, while in RHA-I rats only the 0.5 mg/kg dose impaired PPI at the 80 dB prepulse intensity. DOI slightly decreased locomotion in both strains. Finally, clozapine attenuated the PPI impairment induced by the NMDA receptor antagonist MK-801 only in RLA-I rats.

Conclusions

These results add experimental evidence to the view that RHA-I rats represent a model with predictive and construct validity of some dopamine and 5-HT2A receptors-related features of schizophrenia.

Keywords: Prepulse Inhibition, Activity, Propsychotic drugs, Antipsychotic Drugs, Roman High- and Low-avoidance rats, genetically-based rat model, Predictive Validity, Schizophrenia

Abbreviations

PPI: Prepulse Inhibition

RLA: Roman High-avoidance

RHA: Roman Low-avoidance

1. Introduction

Some very commonly used rodent models of schizophrenia symptoms tend to replicate aspects of the positive symptoms of the disorder, such as hyperactivity or prepulse inhibition (sensorimotor gating) deficits. Prepulse inhibition (PPI; reduction of the startle response elicited by a preceding non-startling stimulus that is shorter and less intense than the startling stimulus) of the acoustic startle response is a measure of sensorimotor gating that is thought to be relevant in some mental disorders like schizophrenia (Kohl et al. 2013), as schizophrenic patients have PPI deficits. Some of the most popular rodent models derive from the similarity of the human effects of psychotomimetic or psychostimulant drugs to the symptoms of schizophrenia. Thus, the administration of psychostimulant or psychotomimetic (hallucinogenic) drugs to rats or mice generally leads to PPI deficits and, very often, induces hyperactivity (Sawa and Snyder, 2002; Powell and Miyakawa, 2006; Jones et al. 2011; Del Río et al. 2014). These drugs may also produce some of the negative and/or cognitive deficits/symptoms of schizophrenia, although their specific profile of effects depends on the main neurochemical mechanisms influenced by each drug class. In general, these symptoms can be reversed either by typical or atypical antipsychotic drugs, which gives pharmacological (predictive) validity to these models (Sawa and Snyder, 2002; Powell and Miyakawa, 2006; Jones et al. 2011; Del Río et al., 2014). The findings that psychotic-like states or symptoms are produced by dopaminergic psychostimulants (dopamine receptor agonists), and that such symptoms are reversed by typical antipsychotic drugs (i.e., D2 dopamine receptor antagonists; Geyer et al. 2001; Jones et al. 2011), support the dopaminergic hypothesis of schizophrenia. On the other hand, the finding that serotoninergic psychotomimetic (serotonin receptor -5-HT2A- agonists) drugs, as well as NMDA (ionotropic glutamate receptor) antagonists also induce psychotic-like symptoms which are reversed by (mostly) atypical antipsychotic drugs (Halberstadt and Geyer, 2013 and Geyer et al., 2001) has provided support to the more modern serotonin and glutamate hypotheses of schizophrenia (Sawa and Snyder, 2002; Powell and Miyakawa, 2006; Jones et al., 2011; Del Río et al., 2014, Halberstadt and Geyer, 2013).

On the other hand, using schizophrenia models in which symptoms (or deficits) are spontaneously present in naïve (untreated) animals is important in order to elucidate whether typical or atypical antipsychotics can ameliorate symptoms in vulnerable subjects (e.g. Geyer et al. 2001). We have recently proposed that one such model, the Roman rat

strains, display genetically determined deficits/impairments in several schizophrenia-related features (Del Rio et al. 2014; Oliveras et al. 2015; Esnal et al. 2016). The Roman High- (RHA) and Low-avoidance (RLA) rat lines/strains (*lines* refers to the outbred, and *strains* refers to the inbred animals) have been selectively and bidirectionally bred for their rapid (RHA) vs. extremely poor (RLA) ability to acquire the two-way active avoidance task (Bignami, 1965; Driscoll and Bättig 1982; Steimer and Driscoll 2003; Escorihuela et al. 1999, Rio-Alamos et al. 2015). There is extensive evidence showing that RHA/RLA rats display differential schizophrenia-relevant features. For instance, the RHA strain/line displays impaired performance in several learning/memory tasks (Aguilar et al., 2002; Driscoll et al., 1995; Escorihuela et al. 1995b; Nil and Bättig, 1981; Oliveras et al. 2015) and enhanced impulsive behavior in the 5-CSRTT and DRL-20 operant tasks (Klein et al., 2014; Moreno et al., 2010; Zeier et al. 1978). RHA rats exhibit deficits in latent inhibition (Fernandez-Teruel et al., 2006; Esnal et al. 2016), augmented mesocortical dopaminergic response to stress (Giorgi et al., 2003), enhanced locomotor as well as mesolimbic dopaminergic sensitization upon the repeated administration of dopaminergic psychostimulants (Corda et al. 2005; Giorgi et al. 2007; Guitart-Masip et al. 2008), a more robust functional tone of nigrostriatal and mesolimbic dopamine neurons (Tournier et al. 2013) and neurochemical and neuromorphological evidence of decreased hippocampal function (Garcia-Falgueras et al. 2012; Meyza et al. 2009; Salles et al. 2001). With regard to other schizophrenia-linked neurotransmitter systems, like serotonin and glutamate, Klein et al. (2014) have shown that RHA-I rats exhibit a dramatically reduced density of mGluR2 receptors in the prefrontal cortex and hippocampus, and increased binding density of 5HT2A receptors in the prefrontal cortex (Klein et al. 2014; Wood et al. 2016), similarly to what has been observed *post mortem* in the brains of schizophrenic patients. Gonzalez-Maeso et al. (2007, 2008) proposed that 5-HT2A and mGlu2 receptors, engage in a functional interaction that plays a crucial role in pre-attentive processes (sensorimotor gating) and in psychotic states. Thus, RHA rats display a series of neurobehavioral traits that resemble some schizophrenia—relevant symptoms or associated neural processes.

On the basis of that evidence, we would expect that the typical antipsychotic haloperidol (a preferential dopamine D2 receptor antagonist) would reverse the PPI deficit of RHA-I rats whereas the dopamine (D1/D2) agonist apomorphine would further impair such a deficit. Moreover, given the above mentioned deficit of mGlu2 receptors in RHA-I rats,

and because these receptors are functionally linked to 5-HT2A receptors (Gonzalez-Maeso et al. 2008), we would also predict that the propsychotic agent DOI (5-HT2A receptor agonist) and the atypical antipsychotic clozapine (5-HT2A receptor antagonist, but also antagonist at many other receptors, including D2 receptors) would be less effective on PPI and locomotion in RHA-I than RLA-I rats. Accordingly, it would also be expected that clozapine would show greater ability to reverse the MK-801-induced impairment of PPI in RLA-I than RHA-I rats. Most of these hypotheses were confirmed by our experimental results.

2. Materials and Methods

2.1 Animals

Animals used for the present studies were male rats of the inbred Roman High- (RHA-I, N = 283) and Low-Avoidance (RLA-I, N = 294) rat strains from the permanent colonies maintained at our laboratory (Medical Psychology Unit, Dept. Psychiatry and Forensic Medicine, School of Medicine, Autonomous University of Barcelona) since 1996. They were approximately 4 months old at the beginning of the experiments (weight range 320-420 g), and were housed in same-sexed pairs in standard (50 × 25 × 14 cm) macrolon cages. They were maintained under a 12:12h light-dark cycle (lights on at 08:00 a.m.), with controlled temperature (22 ± 2 ºC) and humidity (50-70 %) and with free access to food and water. Naïve rats were used for each of the ten experiments described below. Autonomous University of Barcelona and they were carried out in accordance with the European Council Directive (2010/63/EU) and Spanish legislation (RD 53/2013).

2.2 Drugs

Haloperidol, clozapine, DOI (2,5-Dimethoxy-4-iodoamphetamine), MK-801 (dizocilpine) and apomorphine were purchased from Sigma–Aldrich (St. Louis, MO, USA). Haloperidol and Clozapine were dissolved in a small amount of glacial acetic acid and then diluted in distilled water. DOI, apomorphine and MK-801 were dissolved in saline (0.9% NaCl). All the solutions were freshly prepared each day.

2.2.1 Treatments

All the drug doses and their respective vehicles were administered subcutaneously (s.c.) in a volume of 1 ml/kg body weight. Haloperidol (0.1, 0.25, 0.50 or 1 mg/kg) or its vehicle were administered 30 minutes before testing. Apomorphine (0.05 mg/kg) or its vehicle were administered 5 minutes before testing. DOI (0.1, 0.25, 0.50 or 1 mg/kg) or its vehicle were administered s.c. 15 minutes before testing. Clozapine (2.5, 5 or 10 mg/kg) or its vehicle were administered 60 minutes before testing and MK-801 (0.1 mg/kg) or its vehicle were subcutaneously administered 20 minutes before testing.

To assess the effects on locomotor activity of haloperidol, DOI and clozapine, the two lowest doses of each drug used in the PPI experiments were chosen and injected at the same time interval before starting the registration of locomotion as in the PPI studies. The effective dose of apomorphine was selected according to a previous report (Sanna et al. 2014), but the effects of apomorphine on locomotor activity were not addressed here because they have been well characterized in our previous study (Gimenez-Llort et al. 2005). For the experiment with clozapine and MK-801 the doses chosen were based on previous studies (Bast et al. 2000; Bubenikova et al. 2005; Cilia et al. 2010; Hadamitzky et al. 2007) and also on our pilot experiments showing that at a dose 0.1 mg/kg MK-801 reduced significantly %PPI in both Roman strains (data not shown).

2.3 Prepulse inhibition of the acoustic startle response

Four sound-attenuated boxes (SR-Lab Startle Response System, San Diego Inst., San Diego, USA) were used. Each box consists of a Plexiglas cylinder placed on top of a platform with a sensor that detects the intensity of the force made by the rat in each trial. Two speakers placed at 15 cm on each side of the cylinder deliver the acoustic stimuli and a white noise generator provides the background noise (55 dB) throughout the whole session. Each box is lit by a 10 W lamp. The data are transduced by an accelerometer into a voltage which is saved into a computer for further analysis.

The startle session started with a 5 min habituation period in the startle chambers. Then, 10 *pulse-alone* trials (105 dB, 40 ms) were delivered in order to obtain a basal measure of the ASR (BASELINE 1). Next, each one of the 6 different types of trials were randomly administered 10 times (i.e., 60 trials in total):

1) Pulse-alone trials (105 dB 40 ms, BASELINE 2)

2) Prepulses of 65/70/75/80 dB (20 ms) followed by the startle stimulus (105 dB, 40 ms), with an inter-stimulus interval of 100 ms.

3) No stimulus trials (background noise 55 dB)

Finally, in order to measure the habituation to the startle stimulus, 5 *pulse-alone* trials were delivered (BASELINE 3).

The interval between trials was 10-20 s with a mean of 15s. The startle magnitude was recorded for 200ms after the onset of the pulse

The degree of PPI (in percentage) was calculated according to the formula:

$$\%PPI = 100 - \left(\frac{startle\ amplitude\ on\ prepulse\ trials}{startle\ amplitude\ on\ pulse-alone\ trials}\right) \times 100$$

2.4 Locomotor activity assessment

Locomotor activity of RHA-I and RLA-I rats was evaluated with 3 identical plexiglas activity cages (40 x 40 x 40 cm). These cages are equipped with photocell beams that are interfaced with a PC to record the locomotor activity of the rats using program Acti-track (Panlab, Spain).

The procedure used was identical in the four experiments except for the intervals between the injection of each drug and the beginning of the testing session, which were the same as in the respective PPI experiments (see above). There was no previous habituation to the apparatus or the testing room. Locomotor activity was measured as the total photocell beam breaks every 5 minutes for a total of 30 minutes.

2.5 Statistical analysis

Statistical analysis was performed using the *Statistical Package for Social Science* (SPSS, version 17).

To assess the percentage PPI data as the dependent variable we performed repeated measures ANOVAs, with the 4 prepulse intensities as a within-subject factor, and the 2 strains and the administered doses as between-subject factors. To assess the differences in the startle response during pulse-alone trials we performed an ANOVA with Baseline 2 as the dependent variable and the *doses* and *strain* as between-subject factors. For locomotor activity experiments we assessed the distance travelled data as the dependent variable using repeated measures multifactor ANOVAs with *drugs doses* and *strains* as between-subjects factors and 6 time intervals (5 minutes each) as a within-subject factor. *Post hoc* pair wise contrasts with the LSD test were performed on PPI and activity measures after significant *Strain x Treatment* ANOVA effects.

To assess the pharmacological interaction between clozapine and MK-801 we conducted a repeated measures ANOVA with *strain*, *clozapine* and *MK-801* as between-subject factors and the prepulse intensities as a within-subject factor. *Post hoc* pair wise contrasts with the LSD test were performed after significant *Strain x Treatment* ANOVA effects. The threshold for statistical significance was set at $p \leq 0.05$.

The number of animals in each experimental group is indicated in the legends to figures. Only the comparisons between the drug doses and the respective vehicle group are indicated in the figures except for the study with clozapine and MK-801 in which the significant comparisons with the veh/MK-801 group are also indicated.

3.Results

3.1 Effects of antipsychotics on PPI and locomotor activity

3.1.1 Haloperidol (Exps 1-2) effects on PPI

In Exp. 1 (Fig. 1) the repeated measures factorial ANOVA (2 strains x 5 haloperidol doses x 4 prepulse intensities –as within-subject factor-) revealed significant *prepulse intensity* (Huynh-Feldt $F(2.86, 182.86) = 373.91$ $p \leq 0.001$), *prepulse intensity x strain*

and *prepulse intensity x treatment* effects (Huynh-Feldt F(6.11,182.86) = 6.11 $p \leq 0.001$ and F(11.43,182.86) = 3.51 $p \leq 0.001$, respectively). There were also *strain* and *treatment* effects (F (1, 64) = 13.00 $p \leq 0.001$; F (4, 64) = 3.64 $p \leq 0.010$; respectively) (Fig.1A). The *strain* effect is due to the overall better PPI performance of the RLA-I rats, and the *treatment* effect indicates a global increase of PPI with haloperidol.

A two-way ANOVA showed a significant *treatment* effect on baseline startle response (F (4, 64) = 5.21 $p \leq 0.001$), as haloperidol overall decreased this measure in both strains (Fig. 1C).

As a clear trend for an increase of PPI with haloperidol 1 mg/kg was observed in Exp. 1, Exp. 2 was carried out to test whether this dose had differential, strain-dependent effects. Indeed, the repeated measures ANOVA (2 strains x 2 haloperidol doses x 4 prepulse intensities –within subject factor-) showed that the 2nd order interaction (*prepulse intensity x strain x treatment*) was significant (Huynh-Feldt F(2.17,78.23) = 4.91 $p \leq 0.008$) as well as the *strain* and *treatment* effects (F(1,36)= 24.40 $p \leq 0.001$ and F(1,36) = 5.17 $p \leq 0.029$, respectively; Fig. 2A). Further *post hoc* pair wise comparisons, to determine the source of that interaction, revealed that %PPI was increased in RHA-I rats treated with 1 mg/kg haloperidol at 65 and 70 dB intensities whereas no effects were observed in RLA-I rats (Fig. 2A)

Two-way ANOVA on baseline startle response showed no significant differences between strains or treatments in Exp. 2 (Fig. 2C).

In summary, both Exp. 1 and 2 show that RHA-I rats display lower %PPI than RLA-I rats and that haloperidol globally increases %PPI, whereas Exp. 2 shows that haloperidol 1 mg/kg improves %PPI in RHA-I rats without significantly affecting %PPI in the RLA-I strain.

3.1.2 Clozapine (Exp 7) effects on PPI

In Exp. 7 the repeated measures factorial ANOVA (2 strains x 4 clozapine doses x 4 prepulse intensities –as within-subject factor-) revealed a significant interaction between *strain* and *prepulse intensity* (Huynh-Feldt F(2.93,161.37) = 13.19 $p \leq 0.001$). The only

main effect found was the *strain* effect ($F(1,55) = 8.89$ $p \leq 0.004$) (Fig. 7A), overall indicating the better performance of the RLA-I rats (Fig. 2A, Fig. 2B) .

Assessment by two-way ANOVA of the baseline startle yielded significant *strain* and *treatment* effects ($F(1, 55) = 8.70$ $p \leq 0.005$ and $F(3, 55) = 12.19$ $p \leq 0.001$, respectively; Fig. 7C). The *strain* effect is due to the increased startle response of the RLA-I whereas the *treatment* effect reflects the overall reduction of startle response in clozapine-treated rats.

3.1.3 Haloperidol (Exp. 3) and clozapine (Exp. 8) effects on locomotor activity

In Exp. 3 the overall repeated measures ANOVA (2 strains x 3 haloperidol doses x 6 time intervals –within subject factor-) revealed a significant *interval x strain x treatment* interaction effect on locomotion (Huynh –Feldt $F(8.85, 181.33) = 2.40$ $p \leq 0.014$, Fig. 3A, Fig. 3B). Moreover, there was a significant *treatment* effect ($F(2,41) = 14.07$ $p \leq 0.001$, Fig. 3A, Fig. 3B). *Post-hoc* pair wise comparisons performed to dissect that interaction indicated that both haloperidol doses reduced locomotor activity in the first three 5-min intervals in RLA-I rats, whereas the 0.1 mg/kg dose was clearly less effective in RHA-I *vs.* RLA-I rats (Fig. 3A, Fig. 3B). These results show that haloperidol reduced locomotion in both strains but the effect elicited by 0.1 mg/kg haloperidol was more marked in RLA-I rats.

Repeated measures ANOVA (2 strains x 3 clozapine doses x 6 time intervals –within subject factor-) was performed on activity (distance travelled). A significant *treatment x interval* interaction was found (Huynh-Feldt $F(8.98,188.57) = 5.57$ $p \leq 0.001$), as well as *strain* ($F(1,42) = 4.24$ $p \leq 0.046$) and *treatment* ($F(2,42) = 12.87$ $p \leq 0.001$) effects (Fig. 8A, Fig. 8B). The *strain* effect is due to the higher activity exhibited by the RHA-I rats, and the *treatment* effect is due to the global reduction of activity induced by clozapine.

Repeated measures ANOVA for the first 15 minutes of the sessions (3 intervals) for both drugs yielded similar results (data not shown).

3.2 Apomorphine and DOI effects on PPI and locomotor activity

3.2.1 Effects of apomorphine (Exp. 4) on PPI

The repeated measures factorial ANOVA (2 strains x 2 apomorphine doses x 4 prepulse intensities –within subject factor-) revealed a significant *strain x prepulse intensity* effect (Huynh-Feldt $F(2.82,118.22) = 5.19$ $p \leq 0.003$). The interaction *strain x treatment* was also significant ($F(1,42) = 4.46$ $p \leq 0.042$), as well as the *strain* and *treatment* effects ($F(1, 42) = 17.28$ $p \leq 0.001$ and $F(1,42) = 6.92$ $p \leq 0.012$, respectively) (Fig. 4A, Fig. 4B). Post-hoc comparisons to determine the source of the *strain x treatment* interaction revealed that RHA-I rats treated with apomorphine showed a reduction in %PPI at all prepulse intensities, whereas PPI performance of RLA-I rats was not disrupted by apomorphine at any prepulse intensity (Fig. 4A, Fig. 4B).

Assessment by means of two-way ANOVA baseline startle revealed a significant *strain* effect ($F(1,42) = 15.99$ $p \leq 0.001$) and a significant *strain x treatment* interaction ($F(3,78) = 6.50$ $p \leq 0.015$) (Fig. 4C). *Post-hoc* comparisons showed that baseline startle was reduced by apomorphine in RHA-I rats but not in their RLA-I counterparts (Fig. 4C).

3.2.2 Effects of DOI (Exp. 5) on PPI

Repeated measures ANOVA (2 strains x 5 DOI doses x 4 prepulse intensities –within subject factor-), revealed significant *strain* and *treatment* effects ($F(1,78) = 5,70$ $p \leq 0.019$ and $F(4,78) = 4.04$ $p \leq 0.005$,respectively) and also a significant interaction between both factors ($F(4,78) = 2.55$ $p \leq 0.046$) (Fig. 5A).The *post-hoc* comparisons evidenced that 0.5 mg/kg DOI significantly reduced %PPI of RHA-I rats at 80 dB prepulse intensity (see *post-hoc* tests in Fig. 5A, Fig. 5B). In RLA-I rats all DOI doses significantly diminished %PPI at the 70 dB prepulse intensity in a dose-dependent manner (see *post-hoc* comparisons in Fig. 5A, Fig. 5B).

Two-way ANOVA evaluation of the baseline startle revealed a significant *treatment* effect ($F(4, 78) = 2.74$ $p \leq 0.034$), indicating an overall decrease of startle response magnitude induced by DOI in both strains (Fig. 5C).

3.2.3 Effects of DOI (Exp. 6) on locomotor activity

Repeated measures ANOVA (2 strains x 3 DOI doses x 6 time intervals –within subject factor-) revealed a significant *interval x treatment* interaction (Huynh-Feldt $F(9.45,189.07) = 4.12$ $p \leq 0.001$. There were also significant *strain* ($F(1, 40) = 7.52$ $p \leq 0.009$) and *treatment* effects ($F(2, 40) = 3.33$ $p \leq 0.046$) (Fig. 6A, Fig. 6B). The *strain* effect is due to more intense locomotion of the RHA-I rats, while the *treatment* effect is apparently due to the reduction of activity induced by 0.25 mg/kg DOI in both rat strains.

Repeated measures ANOVA for the first 15 minutes of the sessions (3 intervals) yielded similar results (data not shown)

3.3 Clozapine effects on MK-801-induced deficits on PPI (Exp. 9)

Repeated measures ANOVA (2 strains x 2 clozapine doses x 2 MK-801 doses x 4 prepulse intensities –within subject factor-), revealed a significant *prepulse intensity x strain* interaction ($F(3, 216) = 2.82$ $p \leq 0.040$), as well as significant effects of the three main factors (*strain* $F(1,72) = 13.21$ $p \leq 0.001$; *clozapine* $F(1,72) = 4.60$ $p \leq 0.035$; *MK-801* $F(1,72) = 41.93$ $p \leq 0.001$). There was also a significant *strain x clozapine x MK-801* interaction ($F(1,72) = 4.39$ $p \leq 0.040$) (Fig. 9A, Fig 9B).

Post-hoc tests applied to clarify the source of this 2nd order interaction showed that MK-801 impaired PPI in RHA-I rats only at the 80 dB prepulse intensity and this effect was not attenuated by clozapine (see Fig 9A), while the impairment in RLA-Is was observed at all prepulse intensities (Fig. 9A) and it was attenuated by clozapine at the 75 dB intensity (Fig. 9A). Likewise, *post-hoc* comparisons on the *total % PPI* measure (Fig. 9B) showed that MK-801 did not significantly impair total %PPI in RHA-I rats, whereas this measure was impaired in RLA-I rats and clozapine significantly (though partially) attenuated such an impairment (Fig. 9B).

Three-way ANOVA on baseline startle revealed significant *strain* ($F(1,72) = 4.13$ $p \leq 0.046$), *clozapine* ($F(1,72) = 11.98$ $p \leq 0.001$) and *MK-801* ($F(1,72) = 15.90$ $p \leq 0.001$) effects, as baseline startle was overall higher in RLA-I *vs.* RHA-I rats and was decreased by clozapine but enhanced by MK-801 (Fig. 9C).

3.4 Clozapine effects on MK-801-induced hyperactivity (Exp. 10)

Repeated measures ANOVA on activity results (the whole 30-min session) revealed a significant *time interval x clozapine* interaction (Huynh-Feldt F (3.50, 161.06) = 6.53 $p \leq 0.001$). Significant effects of *MK-801* and *clozapine* ($F(1,46) = 21.38$ $p \leq 0.001$ and $F(1,46) = 20.25$ $p \leq 0.001$, respectively) were also found (Fig. 10A, Fig. 10B), indicating that in both strains MK-801 induced a statistically significant hyperactivity while clozapine reduced locomotor activity.

We also conducted a repeated measures ANOVA with the first three intervals (i.e. first 15 min) of the session as within-subject factor. Main effects of *clozapine* and *MK-801* were observed ($F(1,46)$ 28.04 $p \leq 0.001$ and $F(1,46) = 24.22$ $p \leq 0.001$; respectively), as well as a *clozapine x interval* ($F(1.80, 82.63) = 14.39$ $p \leq 0.001$) and a *strain x clozapine x interval* interaction ($F(1.80, 82.63) = 3.27$ $p \leq 0.048$). *Post-hoc* comparisons to dissect this 2nd order (i.e., *strain x clozapine x interval)* interaction on locomotor activity during the first three 5-min (0-5, 6-10, 11-15) intervals showed that MK-801 increased locomotor activity of RHA-I rats during the three 5-min intervals (Fig. 10A), but only during the first 5-min interval in RLA-I rats (Fig. 10B). Moreover, the clozapine + MK-801 treatment produced a more marked attenuation of MK-801-induced hyperactivity in RHA-I (see intervals 0-5 and 6-10 in Fig. 10A) than in RLA-I rats (see interval 0-5 in Fig. 10B).

4. Discussion

The present report provides the first pharmacological characterization of the effects of antipsychotics (haloperidol, a typical antipsychotic, and clozapine, an atypical antipsychotic), propsychotic drugs (DOI and MK-801) and the mixed dopamine D1/D2 receptor agonist apomorphine on PPI and locomotor activity in the Roman rat strains. The main findings are as follows: (1) haloperidol normalized the deficit in PPI showed by RHA-I rats, without affecting PPI in RLA-I rats; moreover, haloperidol reduced locomotor activity more markedly in the RLA-I strain ; (2) clozapine (a 5-HT2A and dopamine D2 receptor antagonist) failed to affect PPI in either strain, but reduced locomotor activity in both strains; (3) apomorphine impaired PPI only in RHA-I rats; (4) the propsychotic drug DOI (5-HT2A receptor agonist) disrupted PPI in RLA-I rats in a

dose-dependent manner while only the 0.5 mg/kg dose impaired PPI in RHA-I rats; (5) DOI moderately decreased locomotor activity in both strains, (6) clozapine significantly antagonized the MK-801-induced PPI deficit only in RLA-I rats, and (7) clozapine appeared to antagonize MK-801-induced hyperactivity more effectively in RHA-I *vs.* RLA-I rats .

Remarkably, a general finding of all PPI experiments was that the total averaged %PPI was consistently lower in RHA-I than in RLA-I rats, which is in agreement with previous studies showing that the RHA-I strain displays a relative deficit in PPI (Oliveras et al. 2015; Esnal et al. 2016; Del Rio et al. 2014) .

4.1. Haloperidol and clozapine effects on PPI and locomotor activity

In order to assess the predictive validity of genetically-based rat models of schizophrenia-related symptoms it is crucial that propsychotic and antipsychotic drugs are tested alone in naïve animals, to evaluate whether they are able to further impair or reverse PPI deficits, respectively (e.g. Geyer et al. 2001). For example, using this approach Hadamitzky et al. (2007) found that the deficits displayed by rats bred for their low scores of PPI could be restored by haloperidol, but not by clozapine. Moreover, Cilia et al. (2010) reported that Brattleboro rats (which have a mutant gene for vasopressin and display impaired PPI) show a significant improvement of PPI upon treatment with haloperidol and atypical antipsychotics like clozapine and risperidone. In the present study we have used this approach in order to gather evidence on the pharmacological validity of the Roman rat strains (which markedly differ in their basal PPI performance) as a model of differential schizophrenia-related features.

We report that haloperidol 1 mg/kg reverses the PPI deficit of RHA-I rats, without affecting RLA-I rats, whereas the atypical antipsychotic clozapine does not affect PPI in either strain. Moreover, haloperidol reduced baseline startle of both strains in Exp. 1 but did not affect it in Exp. 2, while clozapine (Exp. 7) also decreased startle in both strains. The fact that in Exp. 2 haloperidol increased PPI in RHA-I rats in the absence of changes of baseline startle strengthens the idea that the drug genuinely improves sensorimotor gating in this strain (Swerdlow et al. 2000). Conversely, the lack of effects of clozapine might be related to its startle-decreasing effects (in pulse-alone trials) in both strains.

Similar effects were observed by Hadamitzky et al. (2007) in their selectively-bred low-PPI rat line, as they also found that haloperidol but not clozapine improved PPI. The authors attributed the lack of effects of clozapine to its (baseline) startle-inhibiting effects, although they also noted that when this drug has an effect it is often in a narrow dose range (i.e. it is likely to miss an effective dose in a particular study) and that its effects (both on startle and on PPI) vary between different rat strains, with Wistar rats (which are the original strain from which the RHA/RLA lines were derived) being a particularly resistant strain (Hadamitzky et al. 2007).These results are in contrast with previous studies usually showing that typical antipsychotics do not ameliorate PPI deficits while atypical antipsychotics revert PPI deficits (among other negative and cognitive symptoms) in naïve rats (see Geyer et al 2001; but also see Cilia et al. 2010; Feifel et al. 2001; Hadamitzky et al. 2007).

Finally, haloperidol more markedly reduced locomotor activity in RLA-Is than RHA-I rats, while clozapine reduced activity of both rat strains to a similar extent. While there have been no previous studies with clozapine in these strains, the effects of haloperidol on locomotor activity are consistent with previous studies with the Roman rats. For example, Sanna et al. (2014b) reported that haloperidol impaired copulatory behavior in RLA, but not RHA rats. The authors interpreted that these effects may be due to the lowered dopaminergic tone and increased D2 receptor density (Tournier et al. 2013) in the brain of RLA rats, which might make this strain more sensitive than RHA rats to the inhibition of locomotion induced by haloperidol thereby interfering with copulatory behavior. Moreover, in keeping with this hypothesis and with our present results, Eilam and Szechtman (1989) reported that RHA rats are less sensitive than their RLA counterparts to the inhibitory effect on locomotion of quinpirole, which is thought to be mediated by the activation of presynaptic inhibitory D2 autoreceptors.

4.2. Apomorphine and DOI effects on PPI and locomotor activity

The mixed D1/D2 receptor agonist apomorphine and the propsychotic (5-HT2A agonist) DOI globally impaired PPI, in a strain-dependent manner. Thus, the apomorphine-induced impairment was evident only in RHA-I rats, whereas the PPI-disrupting effects of DOI were more robust in RLA-I rats. Moreover, apomorphine decreased startle responses to pulse-alone stimuli in RHA-I rats and a similar trend was observed with

DOI. It might be argued that the disruption of PPI induced by apomorphine in RHA-I rats might be related to the observed reduction of startle responses in that strain; however the PPI-disrupting apomorphine effect is a well-established phenomenon (e.g. Swerdlow et al. 2000), that is sometimes paralleled by a drug-induced reduction of startle responses to pulse-alone trials (e.g. Breier et al. 2010; Swerdlow et al. 2000). As the phenomenon has been replicated in several rat strains and has been shown to be heritable, regardless of whether or not it is sometimes associated to a reduction of startle responses, it is generally considered as solid evidence that apomorphine induces an impairment of sensorimotor gating (e.g. Breier et al. 2010; Swerdlow et al. 2000). Similarly, the PPI-disruptive effect of DOI, also a well-characterized phenomenon (e.g. Farid et al. 2000; Wischhof et al 2012; see below), is present in RLA-I rats at doses devoid of effects on baseline startle response (see Fig. 5B and Fig. 5C). A moderate decrease of locomotor activity was also observed with DOI (as said in *Methods* section, the effects of apomorphine on locomotor activity in both strains were not addressed here because they have been well characterized in our previous study; Gimenez-Llort et al. 2005).

The above results give support to our hypotheses as, based on neurochemical and neuropharmacological evidence of differential central dopaminergic function between both Roman rat lines/strains (see *Introduction*), we predicted that RHA-I rats would be more sensitive to the haloperidol-improving and apomorphine-impairing effects on PPI. In fact, there is mounting evidence supporting the view that the functional tone of nigrostriatal and mesolimbic dopamine neurons is more robust in RHA than RLA rats, and it has been suggested that the lower availability of inhibitory D2 autoreceptors in the cell bodies of nigrostriatal and mesolimbic dopaminergic neurons of RHA rats (Tournier et al. 2013), may account (at least in part) for the behavioral differences between the two rat lines/strains (e.g. Driscoll et al. 2009; Giorgi et al. 2007; Guitart-Masip et al. 2008a,b; Sanna et al. 2013, 2014a,b; Tournier et al. 2013). Together with the finding that RHA rats show increased sensitivity to dopaminergic psychostimulants (Corda et al. 2005; Giorgi et al. 2007; Gimenez-Llort el al. 2005) and to the behavioral and neurochemical sensitization effects of these drugs (Corda et al. 2005; Giorgi et al. 2007; Guitart-Masip et al. 2008a), the results of the present PPI experiments using haloperidol and apomorphine lend further support to the validity and usefulness of RHA-I vs RLA-I rats as a model of some dopamine-related schizophrenia-relevant symptoms.

On the other hand, in keeping with previous reports, DOI significantly disrupted PPI in both strains (e.g. Sipes and Geyer, 1997; Johansson et al., 1995; Padich et al., 1996; Sipes and Geyer, 1995b) and, consistent with our hypothesis, this effect was more pronounced in RLA-I than in RHA-I rats (see Fig. 5). A differential strain-related sensitivity to the PPI-disrupting effect of DOI has also been reported by Wischhof et al. (2012), who observed DOI-induced PPI deficits in Wistar, but not Lister Hooded, rats. Our results also show that RLA-I rats are more sensitive to DOI than their RHA-I counterparts. Several mechanisms may explain these strain-related differences in the effects of DOI on PPI, including drug sensitivity, receptor densities or affinities, as well as the coupling to second messengers (Wischhof et al., 2012). In this context, the recently discovered relationship between 5-HT2A (which are the molecular target of DOI) and mGlu2 receptors (Delille et al., 2012; Gonzalez-Maeso et al. 2008) may also be involved in the contrasting effects mentioned above. Thus, Gonzalez-Maeso et al. (2008) and Delille et al. (2012), among others, have proposed that a receptor complex containing 5-HT2A and mGluR2 integrates serotoninergic and glutamatergic signaling in order to convey sensorimotor information in a proper manner. Furthermore, the finding that these receptors are dysregulated in schizophrenic patients supports the idea that the 5-HT2A/mGluR2 receptor complex plays a major role in the neurochemistry of schizophrenia. Accordingly, the expression of psychotic states by 5-HT receptor agonists and antipsychotic effects by atypical antipsychotic drugs (i.e., 5-HT receptor antagonists) requires the presence of both, 5-HT2A and mGluR2 receptors (Fribourg et al. 2011; Gonzalez-Maeso et al. 2008; Klein et al. 2014). Remarkably, as reported by Klein et al. (2014), RHA-I rats showed no detectable protein levels of mGlu2 receptor protein in the frontal cortex, hippocampus and the striatum, a deficit that is apparently due to a mutation in the grm2 gene (Wood et al. 2016). Thus, in line with the above evidence it seems plausible that the reduced PPI-impairing effects of DOI in RHA-I rats (relative to RLA-Is) may be due to their low cerebral density of mGluR2 receptors. On the other hand, although there were overall decreasing effects of DOI on baseline startle, taking both rat strains together, the impairment of PPI in RLA-I rats was independent of DOI effects on the basal startle responses (see Fig. 5B-C).

Finally, it is noteworthy that both DOI (i.e. 5-HT2A and 5-HT2C agonist) and clozapine (5-HT2A antagonist and antagonist of various 5-HT, DA and other receptors), despite having partially opposite neurochemical mechanisms of action (i.e. agonist and antagonist

of 5-HT2A receptors, respectively), induced decreases of locomotor activity that were similar in both rat strains. At least three possibilities may be proposed to explain this discrepancy: (i) the locomotor activity test is not specific enough to distinguish between opposite drug-receptor interactions, (ii) the neural mechanism(s) of action that lead to the decrease of locomotion may be different from those involved in PPI effects, and (iii) given that RHA-I and RLA-I rats present the above mentioned differences in central 5-HT2A receptor levels, the mechanisms underlying clozapine- and DOI-induced reduction of activity may not involve (at least not preferentially) 5-HT2A receptors.

4.3. Impairment of PPI and hyperactivity induced by MK-801: Reversal by clozapine

It is well established that NMDA receptor antagonists (PCP, MK-801, ketamine) impair PPI in rodents (for review, see Geyer et al. 2001). Most studies indicate that the disruption of PPI induced by PCP can be reversed/attenuated by clozapine in different species (Andreasen et al. 2006; Ballmaier et al. 2001; Linn et al. 2003; Lipina et al. 2005; Swerdlow et al. 1996), but there is comparatively more controversial evidence on the effects of atypical antipsychotics on MK-801-impaired PPI (Bakshi et al. 1994; Bast et al., 2000; Bubenikova et al., 2005; Fijal et al., 2014; Levin et al., 2005, 2007; Zangrando et al., 2013). However, the experimental evidence seems to suggest that the PPI deficits induced by the administration of NMDA antagonists in rodents can be reversed/attenuated by some atypical antipsychotics but not by the typical antipsychotic haloperidol (e.g. Adell et al. 2012; Bubenikova et al., 2005; Jentsch and Roth, 1999). Thus, following the recent finding that RHA-I rats exhibit increased 5-HT2A receptor density (related to their mGluR2 deficit) and to assess whether this characteristic may lead to different pharmaco-behavioral profiles as compared to RLA-I rats, we chose to antagonize MK-801 effects on PPI and locomotion with clozapine.

The results show that MK-801 produced PPI deficits in both rat strains. Interestingly, the MK-801-induced deficit in PPI was more clearly attenuated by clozapine in RLA-I rats. Also, in agreement with previous reports, MK-801 induced a global increase of startle responses in both rat strains (Bakshi et al. 1994; Bast et al., 2000; Bubenikova et al., 2005; Fijal et al., 2014; Levin et al., 2005). As discussed for the effects of DOI on PPI (see above), the finding that clozapine antagonizes the PPI deficit elicited by MK-801

more effectively in RLA-I than RHA-I rats, is consistent with the putative important role of the 5-HT2A/mGluR2 receptorial complex in sensorimotor gating and pre-attentive processes (Gonzalez-Maeso et al., 2008). Thus, because of their dramatic deficit of mGlu2 receptors (Klein et al., 2014), RHA-I rats would be expected to display weaker effects of 5-HT2A-receptor ligands on PPI as compared with their RLA-I counterparts (Gonzalez-Maeso et al. 2008; Fribourg et al., 2011). This prediction is confirmed by the effects on PPI of DOI (Experiment 4) and clozapine + MK-801 (Experiment 9) reported herein.

The finding that MK-801 increases the baseline startle response suggests that this effect may have contributed to the impairment of PPI induced by MK-801 observed in the present study. However, several lines of evidence argue against this hypothesis. First, the PPI-disrupting effect of MK-801 in rats is a well-established phenomenon that has been observed either in the presence (Bakshi et al. 1994; Bast et al., 2000; Bubenikova et al., 2005; Fijal et al., 2014; Levin et al., 2005) or in the absence of increases of the baseline startle response (Bast et al. 2000; Levin et al. 2005, 2007; Zangrando et al. 2013). Second, it is generally assumed that calculating individual PPI as a percentage of each animal's startle response diminishes the influence that changes in startle reactivity have on PPI (e.g. Feifel et al. 2001; Swerdlow et al. 1994; but see also Swerdlow et al. 2000). Third, baseline startle response and %PPI appear to be dissociable, as suggested by manipulations that change baseline startle but not %PPI (e.g. Feifel et al. 2001; Rigdon 1990; Swerdlow et al. 1986), treatments that alter %PPI but not startle (e.g. Feifel et al. 2001; Furuya et al. 1999; Rigdon 1990; Swerdlow et al. 1990b) or interventions that lead to changes of baseline startle and %PPI either in the same or opposite directions (e.g. Acri et al. 1995; al-Amin and Schwarzkopf 1996). Fourth, according to several studies individual startle amplitude and %PPI are not necessarily correlated (Feifel 1999, 2001; Oliveras et al. 2015; Paylor and Crawley 1997), although sometimes they show low positive associations (Logue et al. 1997; Oliveras et al. 2015; Sanchez-Gonzalez et al. 2016). Fifth, although it has been reported that baseline startle tends to be negatively related with %PPI in some studies with rodents and humans (Csomor et al. 2008; Ellenbroek et al. 1995), other studies, using the Roman rat strains as well as the genetically heterogeneous NIH-HS rat stock, have demonstrated just the opposite, i.e. a positive association between baseline startle and %PPI (Del Rio et al. 2014; Esnal et al. 2016; Oliveras et al. 2015; Sanchez-Gonzalez et al. 2016). Sixth, evidence supporting the dissociation of baseline

startle and %PPI come also from the present study, as haloperidol increased %PPI and did not alter startle in RHA-I rats (Exp. 2), apomorphine and DOI reduced %PPI and also baseline startle in RHA-I rats, MK-801 reduced %PPI but increased the baseline startle, while DOI reduced %PPI at doses not affecting startle in RLA-I rats.

Finally, we observed that MK-801 induced a more robust increment in locomotion in RHA-I vs. RLA-I rats, especially during the first 15 min of the activity test, which was also more effectively attenuated by clozapine in the RHA-I strain. One possible explanation for these result is that the larger increment in locomotion elicited by MK-801 in RHA-I *vs.* RLA-I rats could depend on the stimulatory effect of MK-801 on mesolimbic dopaminergic neurons which, under basal conditions, display a more robust physiological tone in RHA vs. RLA rats (see Giorgi et al., 2007, and references therein). In fact, there is evidence suggesting that NMDA antagonists mediate some symptoms, such as hyperactivity and sensorimotor deficits, through their actions on limbic dopaminergic pathways (Meltzer et al. 2011). Specifically, the NMDA antagonist-induced stimulation of DA release in the nucleus accumbens has been suggested to result from decreased mesocortical dopaminergic function, which in turn enhances (i.e., disinhibits) mesolimbic dopaminergic activity (Del Arco et al. 2007; Del Arco and Mora 2008, 2009; Lannes et al. 1991; Jentsch et al. 1998; Zangrando et al. 2013). The different physiological tone of the mesolimbic dopaminergic projections of RHA and RLA rats may also be involved in the more effective antagonism of the MK-801-elicited locomotor activation by clozapine in RHA-I *vs.* RLA-I rats.

4.4. Conclusions

In conclusion, regarding sensorimotor gating the main novel findings of the present study are that (see Table 1), compared to RLA-I, RHA-I rats show higher sensitivity to the improving effects of haloperidol and to the impairing effects of apomorphine on PPI. Conversely, RLA-I rats are more sensitive than their RHA-I counterparts to the PPI-impairing effects of DOI and MK-801, as well to the reversal of MK-801 effect by clozapine.

With regard to locomotor activity, its reduction by haloperidol is more pronounced in RLA-I rats, whereas MK-801-induced hyperactivity and its reversal by clozapine appear to be more marked in RHA-I rats (see Table 1).

The well known between-strain differences in central dopaminergic function (RHA> RLA) may underlie the observed strain- dependent effects of haloperidol and apomorphine on PPI and locomotor activity. As discussed above, these dopaminergic differences may also be involved in the more robust hyperactivity induced by MK-801 (and its reversal by clozapine) in the RHA-I strain.

Moreover, the results obtained with DOI and clozapine + MK-801 on PPI are consistent with the finding that the RHA-I strain has an alteration of the 5-HT2A/mGluR2 complex (i.e., a deficit of mGlu2 receptors and a relative increase of 5-HT2A receptors; Klein et al., 2014; Wood et al., 2016), similar to that observed in schizophrenic patients (Gonzalez-Maeso et al. 2008). Thus, RHA-I rats may represent a model of dopamine- and 5-HT2A/mGluR2-related schizophrenia-relevant features.

Acknowledgements.- This work was supported by grants PSI2013-41872-P (MINECO), 2014SGR-1587 (DGR), "ICREA-Academia 2013" to AF-T and by a grant from the Regional Government of Sardinia (RAS) (L.R.7/2007, Project code no. CRP-59842) to OG. IO is recipient of a PhD FI fellowship (DGR 2014). AS-G is recipient of a PhD FPI fellowship. MAP is a recipient of a PhD fellowship from RAS.

References

Acri JB, Brown KJ, Saah MI, Grunberg NE (1995) Strain and age differences in acoustic startle responses and effects of nicotine in rats. Pharmacol Biochem Behav 50:191–198. doi:10.1016/0091-3057(94)00285-Q

Adell A, Jiménez-Sánchez L, López-Gil X, Romon T (2012) Is the acute NMDA receptor hypofunction a valid model of schizophrenia? Schizophrenia Bull 38:9–14. doi:10.1093/schbul/sbr133

Aguilar R, Escorihuela RM, Gil L, Tobeña A, Fernández-Teruel A (2002) Differences between two psychogenetically selected lines of rats in a swimming pool matching-to-place task: long-term effects of infantilestimulation. Behav Genet 32:127–134. doi:10.1023/A:1015253807488

Al-Amin HA, Schwarzkopf SB (1996) Effects of the PCP analog dizocilpine on sensory gating: potential relevance to clinical subtypes of schizophrenia. Biol Psychiatry 40:744–754.
doi:10.1016/0006-3223(95)00485-8

Andreasen JT, Andersen KK, Nielsen EØ, Mathiasen L, Mirza NR (2006) Nicotine and clozapine selectively reverse a PCP-induced deficit of PPI in BALB/cByJ but not NMRI mice: comparison with risperidone. Behav Brain Res 167:118–127.
doi:10.1016/j.bbr.2005.08.023

Bakshi VP, Swerdlow NR, Geyer MA (1994) Clozapine antagonizes phencyclidine-induced deficits in sensorimotor gating of the startle response. J Pharmacol Exp Ther 271:787–794

Ballmaier M, Zoli M, Mazzoncini R, Gennarelli M, Spano F (2001) Combined alpha 2-adrenergic/D2 dopamine receptor blockade fails to reproduce the ability of clozapine to reverse phencyclidine-induced deficits in prepulse inhibition of startle. Psychopharmacology 159: 105–110.
doi:10.1007/s002130100905

Bast T, Zhang W, Feldon J, White IM (2000) Effects of MK801 and neuroleptics on prepulse inhibition: re-examination in two strainsof rats. Pharmacol Biochem Behav 67:647–658

Bignami G (1965) Selection for high rates and low rates of avoidance conditioning in the rat. Anim Behav 13:221–227.
doi:10.1016/0003-3472(65)90038-2

Breier MR, Lewis B, Shoemaker J, Light G, Swerdlow NR (2010) Sensory and sensorimotor gating-disruptive effects of apomorphine in Sprague Dawley and long Evans rats. Behav Brain Res 208:560– 565.
doi:10.1016/j.bbr.2009.12.037

Bubeníková V, VotavaM, Horáček J, Páleníček T, Dockery C (2005) The effect of zotepine, risperidone, clozapine and olanzapine on MK-801-disrupted sensorimotor gating. Pharmacol Biochem Behav 80:591–596.
doi:10.1016/j.pbb.2005.01.012

Cilia J, Gartlon JE, Shilliam C, Dawson LA, Moore SH, Jones DNC (2010) Further neurochemical and behavioural investigation of Brattleboro rats as a putative model of schizophrenia. J Psychopharmacol 24:407–419.
doi:10.1177/0269881108098787

Corda MG, Piras G, Lecca D, Fernández-Teruel A, Driscoll P, Giorgi O (2005) The psychogenetically selected roman rat lines differ in the susceptibility to develop amphetamine sensitization. Behav Brain Res 157:147–156.
doi:10.1016/j.bbr.2004.06.016

Csomor PA, Yee BK, Vollenweider FX, Feldon J, Nicolet T, Quednow BB (2008) On the influence of baseline startle reactivity on the indexation of prepulse inhibition. Behav Neurosci 122:885–900.
doi:10.1037/0735-7044.122.4.885

Del Arco A, Mora F (2008) Prefrontal cortex-nucleus accumbens interaction: in vivo modulation by dopamine and glutamate in the prefrontal cortex. Pharmacol Biochem Behav 90:226–235.
doi:10.1016 /j.pbb.2008.04.011

Del Arco A, Mora F (2009) Neurotransmitters and prefrontal cortexlimbic system interactions: implications for plasticity and psychiatric disorders. J Neural Transm 116:941–952.
doi:10.1007/s00702- 009-0243-8

Del Arco A, Mora F, Mohammed AH, Fuxe K (2007) Stimulation of D2 receptors in the prefrontal cortex reduces PCP-induced hyperactivity, acetylcholine release and dopamine metabolism in the nucleus accumbens. J Neural Transm 114:185–193. doi:10.1007/s00702-006-0533-3

Del Río C, Oliveras I, Cañete T, Blázquez G, Tobeña A, Fernández-Teruel A (2014) Genetic ratmodels of schizophrenia-relevant symptoms.World J Neurosci 4(3):261–278

Delille HK, Becker JM, Burkhardt S, Bleher B, Terstappen GC, Schmidt M et al (2012) Heterocomplex formation of 5-HT2A-mGlu2 and its relevance for cellular signaling cascades. Neuropharmacology 62:
2184–2191.
doi:10.1016/j.neuropharm.2012.01.010

Driscoll P, Bättig K (1982) Behavioural, emotional and neurochemical profiles of rats selected for extreme differences in active, two-way avoidance performance. In: Lieblich I (ed) Genetics of the brain. Elsevier, Amsterdam, pp 95–123

Driscoll P, Ferré P, Fernández-Teruel A, Tobeña A, Escorihuela RM, Levi de Stein M et al (1995) Effects of prenatal diazepam on two-way avoidance behavior, swimming navigation and brain levels of benzodiazepine-like molecules in male roman high- and lowavoidance rats. Psychopharmacology 122:51–57.
doi:10.1007 /BF02246441

Driscoll P, Fernández-Teruel A, Corda MG, Giorgi O, Steimer T (2009) Some guidelines for defining personality differences in rats. In: Yong-Kyu K (ed) Handbook of behavior genetics. Springer, New York, NY, pp 281–300

Eilam D, Szechtman H (1989) Biphasic effect of D-2 agonist quinpirole on locomotion and movements. Eur J Pharmacol 161:151–157

Ellenbroek BA, GeyerMA, Cools AR (1995) The behavior of APO-SUS rats in animal models with construct validity for schizophrenia. J Neurosci 15:7604–7611

Escorihuela RM, Tobeña A, Fernández-Teruel A (1995) Environmentalenrichment and postnatal handling prevent spatial learning deficits in aged hypoemotional (roman high-avoidance) and hyperemotional (roman low-avoidance) rats. Learning & Memory 2:40–48.
doi:10.1101/lm.2.1.40

Escorihuela RM, Fernández-Teruel A, Gil L, Aguilar R, Tobeña A, Driscoll P (1999) Inbred roman high- and low-avoidance rats: differences in anxiety, novelty-seeking, and shuttlebox behaviors. Physiol Behav 67:19–26.
doi:10.1016/S0031-9384(99)00064-5

Esnal A, Sánchez-González A, Río-Álamos C, Oliveras I, Cañete T, Blázquez G et al (2016) Prepulse inhibition and latent inhibition deficits in roman high-avoidance vs. roman low-avoidance rats: modeling schizophrenia-related features. Physiol Behav 163:267– 273.
doi:10.1016/j.physbeh.2016.05.020

Farid M, Martinez Z A, Geyer MA, Swerdlow NR (2000) Regulation of sensorimotor gating of the startle reflex by serotonin 2A receptors.Ontogeny and strain differences. Neuropsychopharmacology 23: 623–632.
doi:10.1016/S0893-133X(00)00163-9

Feifel D (1999) Individual differences in prepulse inhibition of startle as a measure of individual dopamine function. Behav Neurosci 113: 1020–1029

Feifel D, Priebe K, Shilling PD (2001) Startle and sensorimotor gating in rats lacking CCK-A receptors.Neuropsychopharmacology 24:663– 670.
doi:10.1016/S0893-133X(00)00235-9

Fernández-Teruel A, Blázquez G, Pérez M, Aguilar R, Cañete T, Guitart M et al (2006) Latent inhibition threshold in roman high-avoidance rats: a psychogenetic model of abnormalities in attentional filter? Actas Esp. Psiquiatr 34:257–263

Fijał K, Popik P, Nikiforuk A (2014) Co-administration of 5-HT6 receptor antagonists with clozapine, risperidone, and a 5-HT2A receptor antagonist: ef fects on prepulse inhi bition in rats . Psychopharmacology 231:269–281.
doi:10.1007/s00213-013-3234-2

FribourgM,Moreno JL, Holloway T, Provasi D, Baki L, Mahajan R et al (2011) Decoding the signaling of a GPCR heteromeric complex reveals a unifying mechanism of action of antipsychotic drugs. Cell 147:1011–1023.
doi:10.1016/j.cell.2011.09.055

Furuya Y, Kagaya T, Ogura H, Nishizawa Y (1999) Competitive NMDA receptor antagonists disrupt prepulse inhibition without reduction of startle amplitude in a dopamine receptor-independent manner in mice. Euro J Pharmacol 364:133–140. doi:10.1016/S0014-2999(98)00839-5

Garcia-Falgueras A, Castillo-Ruiz MM, Put T, Tobeña A, Fernández-Teruel A (2012) Differential hippocampal neuron density between inbred roman high- (low anxious) and low-avoidance (high anxious) rats. Neurosci Lett 522:41–46. doi:10.1016/j.neulet.2012.06.011

Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR (2001) Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology 156:117–154.
doi:10.1007/s002130100811

Giménez-Llort L, Cañete T, Guitart-Masip M, Fernández-Teruel A, Tobeña A (2005) Two distinctive apomorphine-induced phenotypes in the roman high- and low-avoidance rats. Physiol Behav 86:458–466.
doi:10.1016/j.physbeh.2005.07.021

Giorgi O, Lecca D, Piras G, Driscoll P, Corda MG (2003) Dissociation between mesocortical dopamine release and fear-related behaviours in two psychogenetically selected lines of rats that differ in coping strategies to aversive conditions. Eur J Neurosci 17:2716–2726.
doi:10.1046/j.1460-9568.2003.02689.x

Giorgi O, Piras G, Corda MG (2007) The psychogenetically selected roman high- and low-avoidance rat lines: a model to study the individual vulnerability to drug addiction. Neurosci Biobehav R 31: 148–163.
doi:10.1016/j.neubiorev.2006.07.008

González-Maeso J, Weisstaub NV, Zhou M, Chan P, Ivic L, Ang R et al (2007) Hallucinogens recruit specific cortical 5-HT(2A) receptormediated signaling pathways to affect behavior. Neuron 53:439–452.
doi:10.1016/j.neuron.2007.01.008

González-Maeso J, Ang RL, Yuen T, Chan P, Weisstaub NV, López-Giménez JF et al (2008) Identification of a serotonin/glutamate receptor complex implicated in psychosis. Nature 452:93–97.
doi:10.1038/nature06612

Guitart-Masip M, Johansson B, Cañete T, Fernández-Teruel A, Tobeña A, Terenius L, Giménez-Llort L (2008a) Regional adaptations in PSD-95, NGFI-A and secretogranin gene transcripts related to vulnerability to behavioral sensitization to amphetamine in the roman rat strains . Neuroscience 151:195–208.
doi:10.1016/j.neuroscience.2007.09.072

Guitart-Masip M, Johansson B, Fernández-Teruel A, Tobeña A, Giménez-Llort L (2008b) Divergent effect of the selective D3 receptor agonist PD-128,907 on locomotor activity in roman high- and low-avoidance rats: relationship to NGFI-A gene expression in the Calleja islands. Psychopharmacology 196:39–49.
doi:10.1007/s00213-007-0925-6

HadamitzkyM, Harich S, Koch M, Schwabe K (2007) Deficient prepulse inhibition induced by selective breeding of rats can be restored by the dopamine D2 antagonist haloperidol. Behav Brain Res 177:364–367.
doi:10.1016/j.bbr.2006.11.037

Halberstadt AL, Geyer MA (2013) Serotonergic hallucinogens as translational models relevant to schizophrenia. Int J Neuropsychoph 16: 2165–2180.
doi:10.1017/S1461145713000722

Jentsch JD, Roth RH (1999) The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 20:201–225.
doi:10.1016/S0893-133X(98)00060-8

Jentsch JD, Taylor JR, Roth RH (1998) Subchronic phencyclidine administration increases mesolimbic dopaminergic system responsivity and augments stress- and psychostimulant-induced hyperlocomotion. Neuropsychopharmacology 19:105–113.
doi:10.1016/S0893-133X(98)00004-9

Johansson C, Jackson DM, Zhang J, Svensson L (1995) Prepulse inhibition of acoustic startle, a measure of sensorimotor gating: effects of antipsychotics and other agents in rats. Pharmacol Biochem Behav 52:649–654.
doi:10.1016/0091-3057(95)00160-X

Jones CA,Watson DJG, Fone KCF (2011) Animal models of schizophrenia. Brit J Pharmacol 164:1162–1194.
doi:10.1111/j.1476-5381.2011.01386.x

Klein AB, Ultved L, Adamsen D, Santini MA, Tobeña A, Fernández-Teruel A et al (2014) 5-HT(2A) and mGlu2 receptor binding levels are related to differences in impulsive behavior in the roman low- (RLA) and high- (RHA) avoidance rat strains. Neuroscience 263:36–45.
doi:10.1016/j.neuroscience.2013.12.063

Kohl S, Heekeren K, Klosterkötter J, Kuhn J (2013) Prepulse inhibition in psychiatric disorders–apart from schizophrenia. J Psychiatr Res 47: 445–452.
doi:10.1016/j.jpsychires.2012.11.018

Lannes B, Micheletti G, Warter JM, Kempf E, Di Scala G (1991) Behavioural, pharmacological and biochemical effects of acute and chronic administration of ketamine in the rat. Neurosci Lett 128:177–181

Levin ED, Petro A, Caldwell DP (2005) Nicotine and clozapine actions on pre-pulse inhibition deficits caused by N-methyl-D-aspartate (NMDA) glutamatergic receptor blockade. Prog Neuro-Psychoph 29:581–586.
doi:10.1016/j.pnpbp.2005.01.012

Levin ED, Caldwell DP, Perraut C (2007) Clozapine treatment reverses dizocilpine-induced deficits of pre-pulse inhibition of tactile startle response. Pharmacol Biochem Behav 86:597–605.
doi: 10.1016/j.pbb.2007.02.005

Linn GS, Negi SS, Gerum SV, Javitt DC (2003) Reversal of phencyclidine-induced prepulse inhibition deficits by clozapine in monkeys. Psychopharmacology 169:234–239.
doi:10.1007/s00213- 003-1533-8

Lipina T, Labrie V, Weiner I, Roder J (2005) Modulators of the glycine site on NMDA receptors, D-serine and ALX 5407, display similar beneficial effects to clozapine in mouse models of schizophrenia. Psychopharmacology 179:54–67.
doi:10.1007/s00213-005-2210-x

Logue SF, Owen EH, Rasmussen DL,Wehner JM (1997) Assessment of locomotor activity, acoustic and tactile startle, and prepulse inhibition of startle in inbred mouse strains and F1 hybrids: Implications of genetic background for single gene and quantitative trait locianalyses. Neuroscience 80:1075–1086
doi:10.1016/S0306-4522(97)00164-4

Meltzer HY, Horiguchi M, Massey BW (2011) The role of serotonin in the NMDA receptor antagonist models of psychosis and cognitive impairment. Psychopharmacology 213:289–305.
doi:10.1007/s00213-010-2137-8

Meyza KZ, Boguszewski PM, Nikolaev E, Zagrodzka J (2009) Diverse sensitivity of RHA/Verh and RLA/Verh rats to emotional and spatial aspects of a novel environment as a result of a distinct pattern of neuronal activation in the fear/anxiety circuit. Behav Genet 39:48–61.
doi:10.1007/s10519-008-9234-z

Moreno M, Cardona D, Gómez MJ, Sánchez-Santed F, Tobeña A, Fernández-Teruel A et al (2010) Impulsivity characterization in the roman high- and low-avoidance rat strains: behavioral and neurochemical differences. Neuropsychopharmacology 35:1198–1208. doi:10.1038/npp.2009.224

Nil R, Bättig K (1981) Spontaneous maze ambulation and Hebb-Williams learning in roman high-avoidance and roman low-avoidance rats. Behav Neural Biol 33:465–475. doi:10.1016/S0163-1047(81)91833-1

Oliveras I, Río-Álamos C, Cañete T, Blázquez G, Martínez-Membrives E, Giorgi O et al (2015) Prepulse inhibition predicts spatial working memory performance in the inbred roman high- and low-avoidance rats and in genetically heterogeneous NIH-HS rats: relevance for studying pre-attentive and cognitive anomalies in schizophrenia. Front Behav Neurosci 9:213.
doi:10.3389/fnbeh.2015.00213

Padich RA, McCloskey TC, Kehne JH (1996) 5-HT modulation of auditory and visual sensorimotor gating: II. Effects of the 5-HT2A antagonist MDL 100,907 on disruption of sound and light prepulse inhibition produced by 5-HT agonists in Wistar rats. Psychopharmacology 124:107–116

Paylor R, Crawley JN (1997) Inbred strain differences in prepulse inhibition of themouse startle response. Psychopharmacology 132:169–180.
doi:10.1007/s002130050333

Powell CM, Miyakawa T (2006) Schizophrenia-relevant behavioral testing in rodent models: a uniquely human disorder? Biol Psychiat 59: 1198–1207.
doi:10.1016/j.biopsych.2006.05.008

Rigdon GC (1990) Differential effects of apomorphine on prepulse inhibition of acoustic startle reflex in two rat strains. Psychopharmacology 102:419–421.
doi:10.1007/BF02244115

Río-Álamos C, Oliveras I, Cañete T, Blázquez G, Martínez-Membrives E, Tobeña A, Fernández-Teruel A (2015) Neonatal handling decreases unconditioned anxiety, conditioned fear, and improves two-way avoidance acquisition: a study with the inbred roman high (RHA-I)- and low-avoidance (RLA-I) rats of both sexes. Front Behav Neurosci 9:174.
doi:10.3389/fnbeh.2015.00174

Sallés J, López de Jesús M, Goñi O, Fernández-Teruel A, Driscoll P, Tobeña A, Escorihuela RM (2001) Transmembrane signaling through phospholipase C in cortical and hippocampal membranes of psychogenetically selected rat lines. Psychopharmacology 154: 115–125.
doi:10.1007/s002130000621

Sánchez-González A, Esnal A, Río-Álamos C, Oliveras I, Cañete T, Blázquez G et al (2016) Association between prepulse inhibition of the startle response and latent inhibition of two-way avoidance acquisition: a study with heterogeneous NIH-HS rats. Physiol Behav 155:195–201.
doi:10.1016/j.physbeh.2015.12.011

Sanna F, Corda MG, Melis MR, Piludu MA, Löber S, Hübner H et al (2013) Dopamine agonist-induced penile erection and yawning: a comparative study in outbred roman high- and low-avoidance rats. Pharmacol Biochem Behav 109:59–66.
doi:10.1016/j.pbb.2013.05.002

Sanna F, Corda MG, Melis MR, Piludu MA, Giorgi O, Argiolas A (2014a) Male roman high and low avoidance rats show different patterns of copulatory behaviour: comparison with Sprague Dawley rats. Physiol Behav 127:27–36.
doi:10.1016/j.physbeh.2014.01.002

Sanna F, Piludu MA, Corda MG, Argiolas A, Giorgi O, Melis MR (2014b) Dopamine is involved in the different patterns of copulatory behaviour of roman high and low avoidance rats: studies with apomorphine and haloperidol. Pharmacol Biochem Behav 124:211–219.
doi: 10.1016/j.pbb.2014.06.012

Sawa A, Snyder SH (2002) Schizophrenia: diverse approaches to a complex disease. Science 296:692–695.
doi:10.1126/science.1070532

Sipes TE, Geyer MA (1995) DOI disruption of prepulse inhibition of startle in the rat is mediated by 5-HT(2A) and not by 5-HT(2C) receptors. Behav Pharmacol 6:839–842

Sipes TE, GeyerMA (1997) DOI disrupts prepulse inhibition of startle in rats via 5-HT2A receptors in the ventral pallidum. Brain Res 761: 97–104.
doi:10.1016/S0006-8993(97)00316-8

Steimer T, Driscoll P (2003) Divergent stress responses and coping styles in psychogenetically selected roman high-(RHA) and low-(RLA) avoidance rats: behavioural, neuroendocrine and developmental aspects. Stress 6:87–100. doi:10.1080/1025389031000111320

Swerdlow NR, Braff DL, GeyerMA, Koob GF (1986) Central dopamine hyperactivity in rats mimics abnormal acoustic startle response in schizophrenics. Biol Psychiatry 21:23–33.
doi:10.1016/0006-3223(86)90005-3

Swerdlow NR, Mansbach RS, Geyer MA, Pulvirenti L, Koob GF, Braff DL (1990) Amphetamine disruption of prepulse inhibition of acoustic startle is reversed by depletion of mesolimbic dopamine. Psychopharmacology 100:413–416.
doi:10.1007/BF02244616

Swerdlow NR, Braff D, TaiidN,GeyerMA(1994) Assessing the validity an animal model of sensorimotor gating deficits in schizophrenia. Gen Psychiatry 51: 139–154. doi:10/archpsyc.1994.03950020063007

Swerdlow N, Bakshi V, Geyer M (1996) Seroquel restores sensorimotor gating in phencyclidine-treated rats. J Pharmacol Exp Ther 1290–1299

Swerdlow NR, Braff DL, Geyer MA (2000) Animal models of sensorimotor gating: what we know, what we think we know, what we hope to know soon. Behav Pharmacol 11:185–204

Tournier BB, Steimer T,Millet P, Moulin-Sallanon M, Vallet P, Ibañez Ginovart N (2013) Innately low D2 receptor availability is associated with high novelty-seeking and enhanced behavioural sensitization to amphetamine. Int J Neuropsychopharmacol 16:1819–34
doi:10.1017/S1461145713000205

Wischhof L, Aho HEA, KochM(2012) DOI-induced deficits in inhibition in Wistar rats are reversed by mGlu2/3 receptor stimulation. Pharmacol Biochem Behav 102:6–12. doi:10.1016/pbb.2012.03.011

Wood CM, Nicolas CS, Choi SL, Roman E, Nylander I, Fernández-Teruel A. et al (2016) Prevalence and influence of cys407* Grm2 mutation in Hannover-derived Wistar rats: mGlu2 receptor loss links to intake, risk taking and emotional behaviour. Neuropharmacology 115:138-148
doi:10.1016/j.neuropharm.2016.03.020

Zangrando J, Carvalheira R, Labbate G, Medeiros P, Longo BM,Thomas L, Silva RCB (2013) Atypical antipsychotic olanzapine reversed deficit on prepulse inhibition of the acoustic startle produced by microinjection of dizocilpine (MK-801) into inferior colliculus in rats. Behav Brain Res 257:77–82.
doi:10.1016/bbr.2013.09.018

Zeier H, Baettig K, Driscoll P (1978) Acquisition of DRL-20 behavior male and female, roman high- and low-avoidance rats. Physiol Behav 20:791–793.
doi:10.1016/0031-9384(78)90307-4

Legends to figures

Figure 1. A) Mean prepulse inhibition (± S.E.M.) of RHA-I and RLA-I rats is shown for each prepulse intensity and haloperidol doses. B) Mean prepulse inhibition (averaged for the four intensities ± S.E.M.) is shown for all the treatments in RHA-I and RLA-I . C) Mean ± S.E.M. of the startle response magnitude in BASELINE 2 for each treatment administered to the RHA-Is and RLA-Is. S : strain effect (ANOVA); T: treatment effect (ANOVA); S x Int: strain x prepulse intensity interaction (ANOVA); T x Int : Treatment x prepulse intensity interaction (ANOVA). The number of rats in each experimental group was as follows: RHA-I, vehicle, n=6; 0.1 mg/kg, n=6; 0.25 mg/kg, n=6; 0.5 mg/kg, n=6; 1 mg/kg, n=10; RLA-I, vehicle, n=8; 0.1 mg/kg, n=7; 0.25 mg/kg, n=7; 0.5 mg/kg, n=8; 1 mg/kg, n=10.

Figure 2. A) Mean prepulse inhibition (± S.E.M.) of RHA-I and RLA-I rats is shown for each prepulse intensity and haloperidol doses (n=10/group in both strains). B) Mean prepulse inhibition (averaged for the four intensities ± S.E.M.) is shown for all the treatments of RHA-I and RLA-I rats. C) Mean ± S.E.M. of the baseline startle response for each treatment administered to the RHA-I and RLA-I rats. T: Treatment effect (ANOVA) S x T x Int: strain x treatment x prepulse intensity 2nd order interaction (ANOVA). *p < 0.05 *vs.* the prepulse intensity-matched and vehicle-treated RHA-I group (LSD test after significant S x T x Int effect in ANOVA).

Figure 3. A) Distance (± S.E.M.) travelled in each 5-min interval of the locomotor activity test, by RHA-I rats and haloperidol doses (vehicle, n=8; 0.1 mg/kg, n=8; 0.25 mg/kg, n=7) . B) Distance (± S.E.M.) travelled in each 5-min interval of the locomotor activity test, by RLA-I rats and haloperidol doses (n=8/group). C) Total distance (± S.E.M.) travelled by RHA-I and RLA-I rats in the 30-min activity session. A and B: ***p<0.001, **p<0.01, *p<0.05 both doses *vs.* the vehicle-treated group (V); ## p<0.01, #p<0.05 *vs.*the vehicle-treated group (dose indicated). T: Treatment effect (ANOVA); S xT x I: strain x treatment x time interval 2nd order interaction (ANOVA).C: ***p<0.001, **p<0.01, *vs.* vehicle-treated group (*post-hoc* LSD test following significant S xT x I effect in ANOVA).

Figure 4. A) Mean prepulse inhibition (± S.E.M.) of RHA-I and RLA-I rats is shown for each prepulse intensity and apomorphine dose (RHA-I: vehicle n=15; 0.05 mg/kg, n=9; RLA-I: vehicle, n=15; 0.05 mg/kg, n=7). B) Mean prepulse inhibition (averaged for the four intensities ± S.E.M.) is shown for both treatments and both strains. C) Mean ± S.E.M. of the baseline startle response for each treatment administered to the RHA-I and RLA-I rats. S: strain effect (ANOVA); T: treatment effect (ANOVA); S x T: strain x treatment interaction (ANOVA); S x Int: strain x prepulse intensity interaction (ANOVA). **p < 0.01, *p < 0.05 (0.05 mg *vs.*the respective vehicle group (*post hoc* pair wise contrasts with the LSD test following significant S x T effect from ANOVA).

Figure 5. A) Mean prepulse inhibition (± S.E.M.) of RHA-I and RLA-I rats is shown for each prepulse intensity and DOI doses. B) Mean prepulse inhibition (averaged for the four intensities ± S.E.M.) is shown for all the treatments in RHA-I and RLA-I rats. C) Mean ± S.E.M. of baseline startle for each treatment administered to the RHA-Is and RLA-Is. S: strain effect (ANOVA); T: treatment effect (ANOVA); S x T: strain x treatment interaction (ANOVA).**p < 0.01, *p < 0.05 *vs.*the respective vehicle group (*post hoc* pair wise contrasts with the LSD test following significant S x T effect from ANOVA). The number of rats in each experimental group was as follows: RHA, vehicle, n=14; 0.1mg/kg, n=8; 0.25 mg/kg, n=8; 0.50 mg/kg, n=6; 1 mg/kg, n=8; RLA-I, vehicle, n=16; 0.1mg/kg, n=8; 0.25 mg/kg, n=6; 0.50 mg/kg, n=8; 1 mg/kg, n=6.

Figure 6. A) Distance (± S.E.M.) travelled in each 5-min interval of the activity test, by RHA-I rats and DOI doses (vehicle, n=8; 0.1mg/kg, n=7; 0.25 mg/kg, n=7). B) Distance (± S.E.M.) travelled in each 5-min interval of the activity test, by RLA-I rats and DOI doses (n=8 rats/group). C) Total distance (± S.E.M.) travelled by RHA-I and RLA-I rats in the 30-min activity session. S: strain effect (ANOVA); T: treatment effect (ANOVA); T x I: treatment x time interval interaction (ANOVA).

Figure 7. A) Mean prepulse inhibition (± S.E.M.) of RHA-I and RLA-I rats is shown for each prepulse intensity and clozapine dose. B) Mean prepulse inhibition (averaged for the four intensities ± S.E.M.) is shown for all the treatments in RHA-I and RLA-I rats. C) Mean ± S.E.M. baseline startle response for each treatment administered to the RHA-I and RLA-I rats. S: strain effect (ANOVA); T: treatment effect (ANOVA); S x Int: strain x prepulse intensity interaction (ANOVA). The number of rats in each experimental group was as follows: RHA, vehicle, n=9; 2.5 mg/kg, n=7; 5 mg/kg, n=8; 10 mg/kg, n=8; RLA-I, vehicle, n=8; 2.5 mg/kg, n=7; 5 mg/kg, n=8; 10 mg/kg, n=8.

Figure 8. A) Distance (± S.E.M.) travelled in each 5-min interval of the locomotor activity test, by RHA-I rats and clozapine doses (n=8 rats/group). B) Distance (± S.E.M.) travelled in each 5-min interval of the activity test, by RLA-I rats and clozapine doses (n=8 rats/group). C) Total distance (± S.E.M.) travelled by RHA-I and RLA-I rats in the 30-min activity session. S: strain effect (ANOVA); T: treatment effect (ANOVA); S x I: strain x time interval interaction (ANOVA).

Figure 9. A) Mean prepulse inhibition (± S.E.M.) of RHA-I and RLA-I rats is shown for each prepulse intensity and each treatment combination. B) Mean prepulse inhibition (averaged for the four intensities ± S.E.M.) is shown for all the treatments in RHA-I and RLAI rats. C) Mean ± S.E.M. of baseline startle response for each treatment administered to the RHA-Is and RLA-Is. S: strain effect (ANOVA); CLZ: clozapine effect (ANOVA); MK-801: MK-801 effect (ANOVA); S x CLZ x MK-801: strain x clozapine x MK-801 (ANOVA). *** p<0.001, **p < 0.01, *p < 0.05 vs vehicle-treated group; ##p<0.01, vs veh/MK-801 (post-hoc LSD test, following significant S x CLZ x MK-801 effect in ANOVA). The number of rats in each experimental group was as follows: RHA-I, veh/veh, n=11; veh/MK-801, n= 9; CLZ/veh, n=8; CLZ/MK-801, n=8; RLA-I, veh/veh , n=14; veh/MK-801, n=10; CLZ/veh, n=10; CLZ/MK-801, n=10.

Figure 10. A) Distance (± S.E.M.) travelled in each 5–min interval of the activity test, by RHA-I rats and treatment combinations (n=6 rats/group). B) Distance (± S.E.M.) travelled in each 5-min interval of the activity test, by RLA-I rats and treatment combinations (veh/veh, n=7; veh/MK-801, n=7; CLZ/veh, n=7; CLZ/MK-801, n=8). C) Total distance (± S.E.M.) travelled by RHA-I and RLA-I rats in the first15-min of the activity session. Total distance (± S.E.M.) travelled by RHA-I and RLA-I rats in the 30-min of the activity session. D) CLZ: clozapine effect (ANOVA); MK-801: MK-801 effect (ANOVA); CLZ x I: clozapine x time interval interaction (ANOVA); S x CLZ x I. Strain x clozapine x time interval interaction. ** $p<0.01$, *$p<0.05$ *vs.* veh/veh group ; ## $p<0.01$, #$p<0.05$ between CLZ/MK-801 and veh/MK-801 groups (*post hoc* pair wise contrasts with the LSD test following significant S x CLZ x I effect from ANOVA).

Figure 1

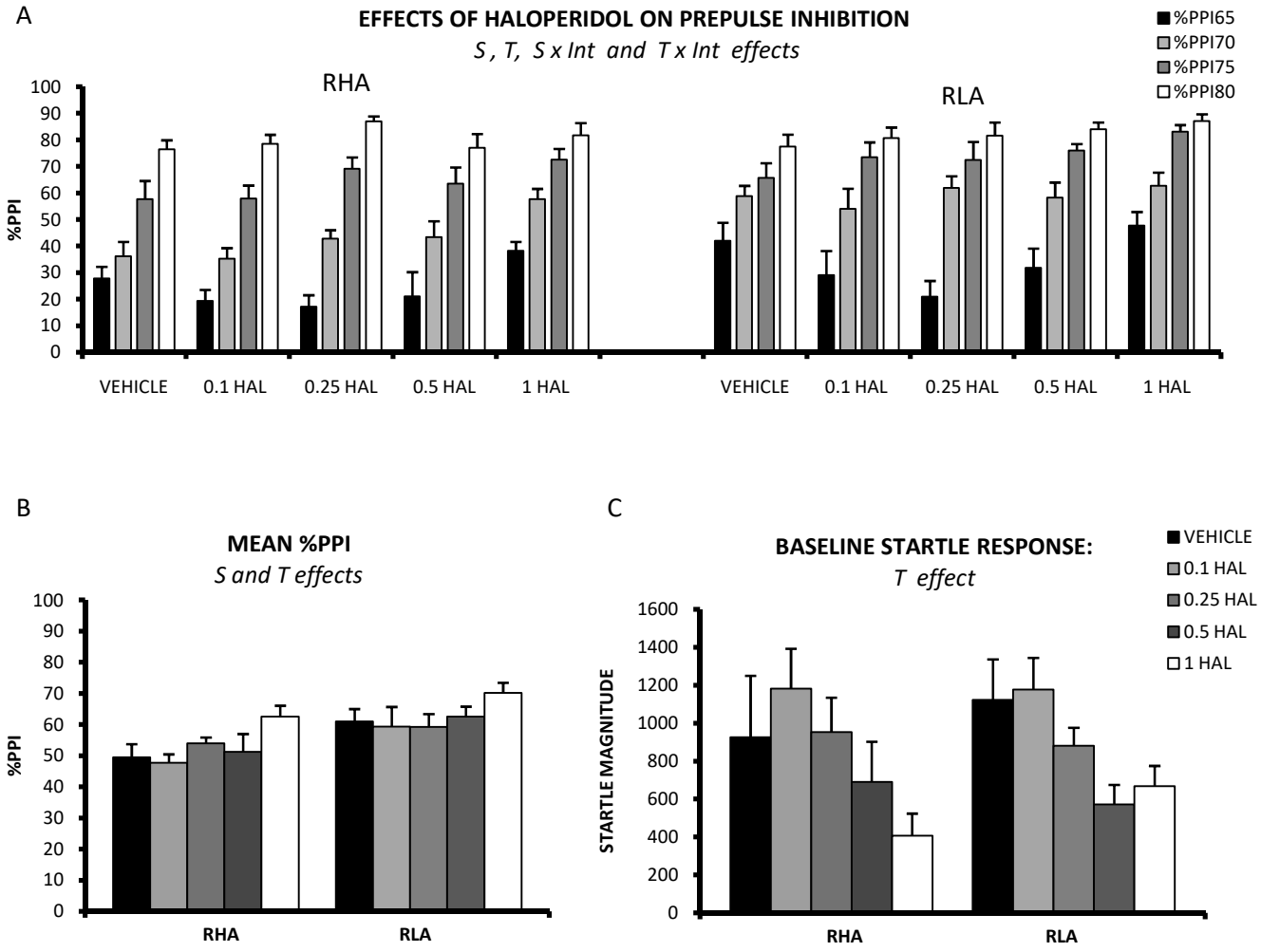

A
EFFECTS OF HALOPERIDOL ON PREPULSE INHIBITION
S , T, S x Int and T x Int effects
%PPI65
%PPI70
%PPI75
%PPI80
RHA
RLA
%PPI
100
90
80
70
60
50
40
30
20
10
0
VEHICLE
0.1 HAL
0.25 HAL
0.5 HAL
1 HAL
VEHICLE
0.1 HAL
0.25 HAL
0.5 HAL
1 HAL
B
MEAN %PPI
S and T effects
%PPI
100
90
80
70
60
50
40
30
20
10
0
RHA
RLA
C
BASELINE STARTLE RESPONSE:
T effect
VEHICLE
0.1 HAL
0.25 HAL
0.5 HAL
1 HAL
STARTLE MAGNITUDE
1600
1400
1200
1000
800
600
400
200
0
RHA
RLA

Figure 2

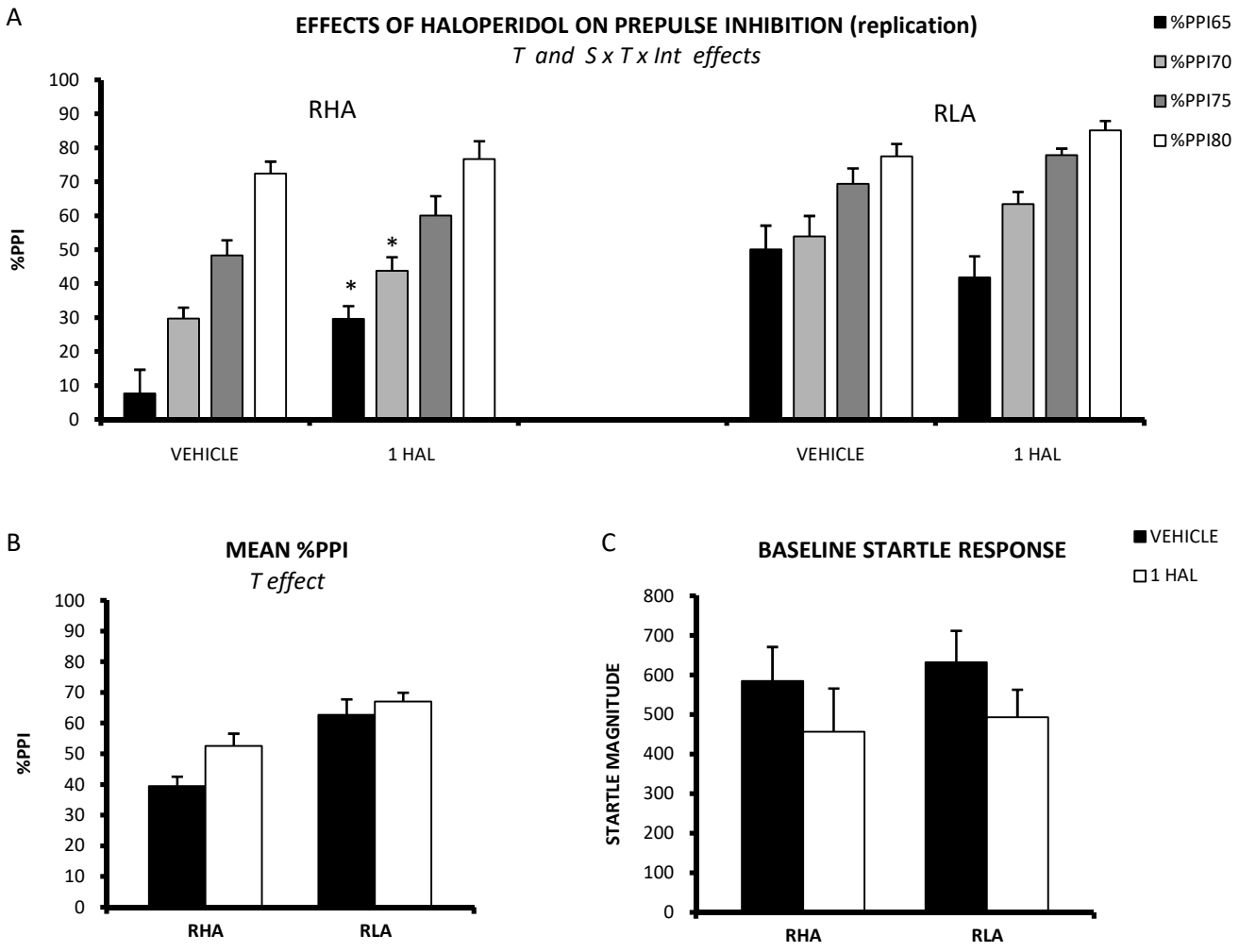

A
EFFECTS OF HALOPERIDOL ON PREPULSE INHIBITION (replication)
T and S x T x Int effects
%PPI65
%PPI70
%PPI75
%PPI80
%PPI
RHA
RLA
VEHICLE
1 HAL
VEHICLE
1 HAL
B
MEAN %PPI
T effect
%PPI
RHA
RLA
C
BASELINE STARTLE RESPONSE
VEHICLE
1 HAL
STARTLE MAGNITUDE
RHA
RLA

Figure 3

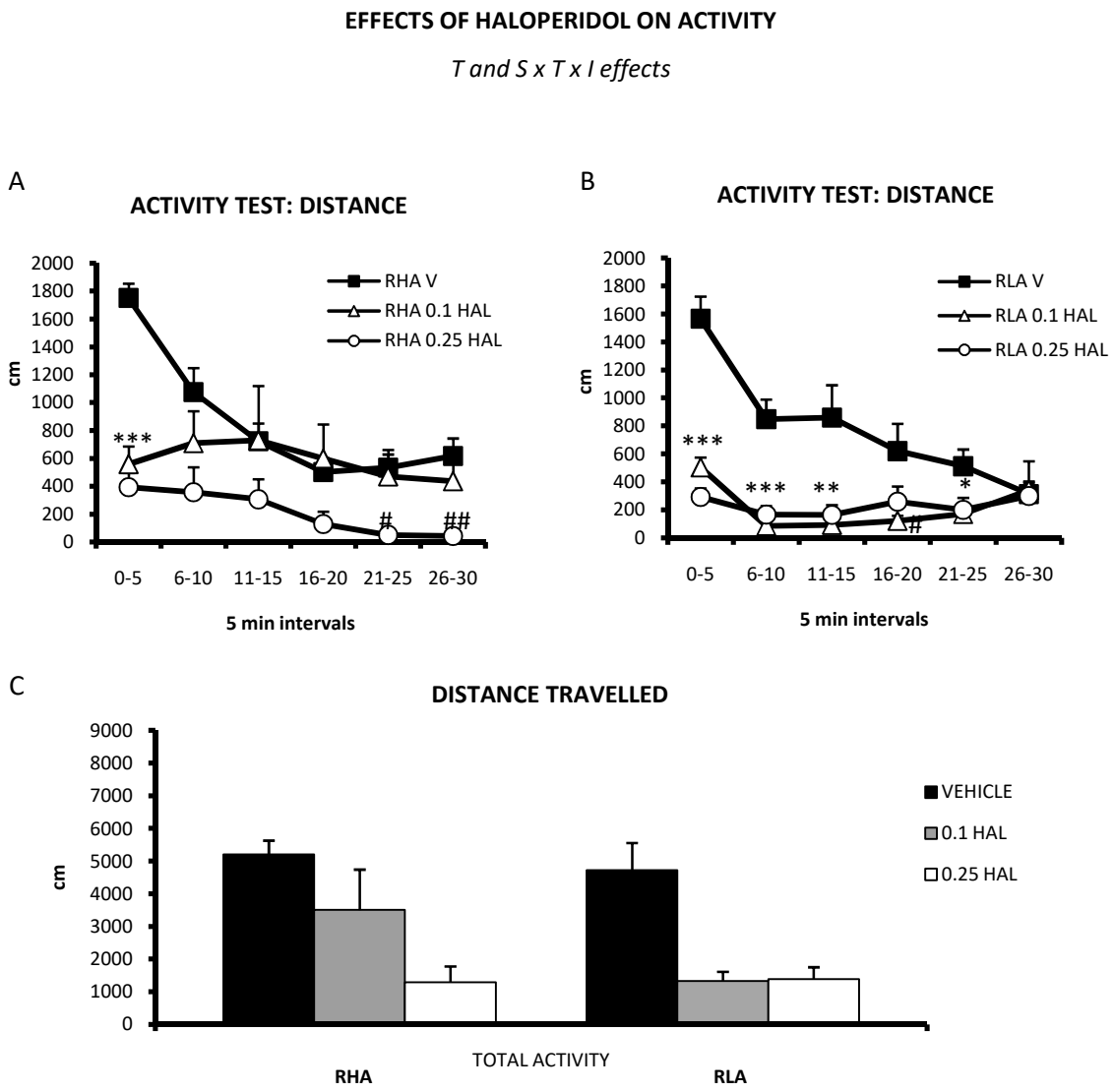

EFFECTS OF HALOPERIDOL ON ACTIVITY
T and S x T x I effects
A
ACTIVITY TEST: DISTANCE
RHA V
RHA 0.1 HAL
RHA 0.25 HAL
cm
5 min intervals
0-5
6-10
11-15
16-20
21-25
26-30
B
ACTIVITY TEST: DISTANCE
RLA V
RLA 0.1 HAL
RLA 0.25 HAL
C
DISTANCE TRAVELLED
VEHICLE
0.1 HAL
0.25 HAL
TOTAL ACTIVITY
RHA
RLA

Figure 4

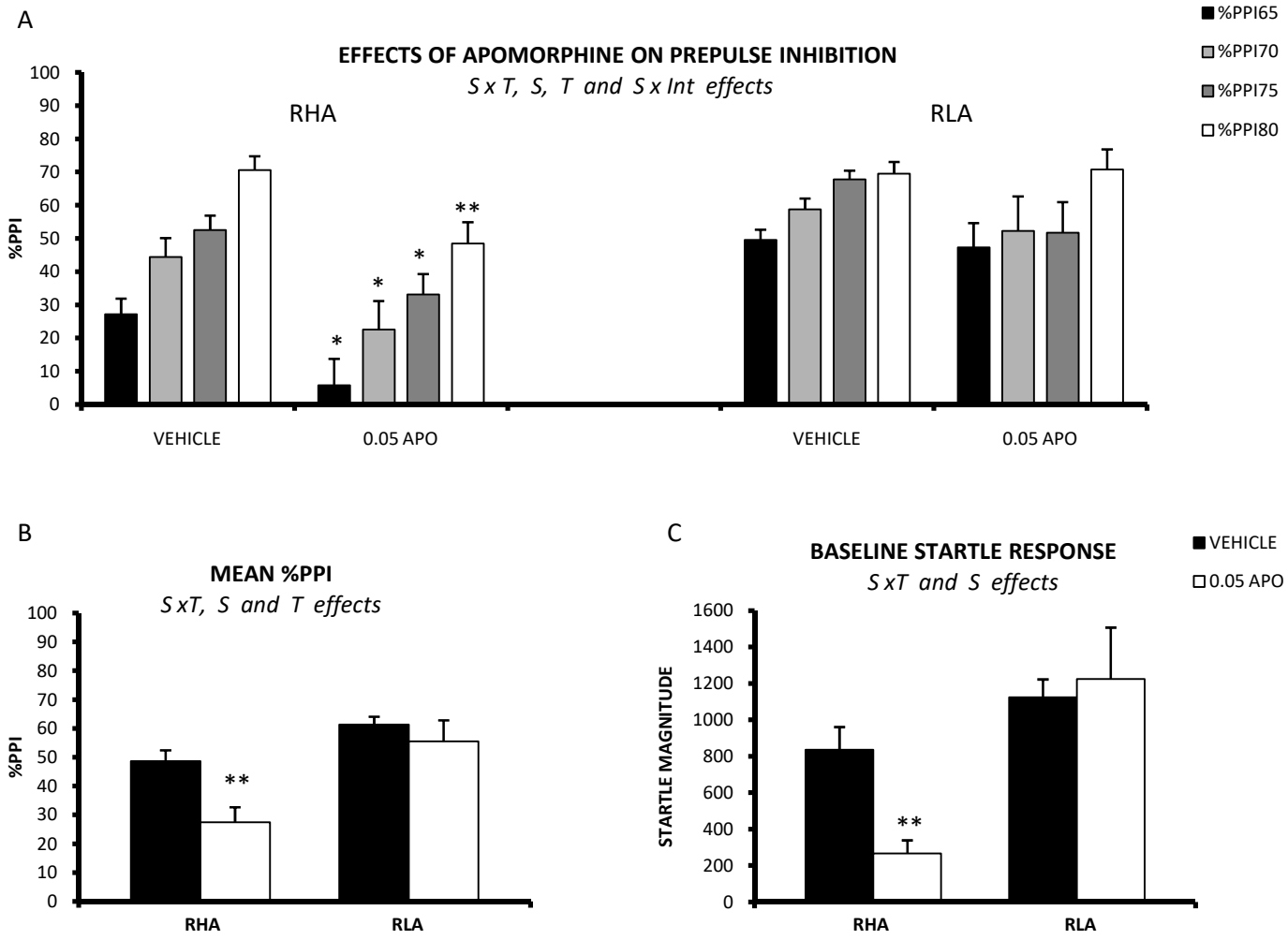

A
EFFECTS OF APOMORPHINE ON PREPULSE INHIBITION
S x T, S, T and S x Int effects
RHA
RLA
%PPI
%PPI65
%PPI70
%PPI75
%PPI80
VEHICLE
0.05 APO
VEHICLE
0.05 APO
B
MEAN %PPI
S xT, S and T effects
%PPI
RHA
RLA
C
BASELINE STARTLE RESPONSE
S xT and S effects
STARTLE MAGNITUDE
VEHICLE
0.05 APO
RHA
RLA

Figure 5

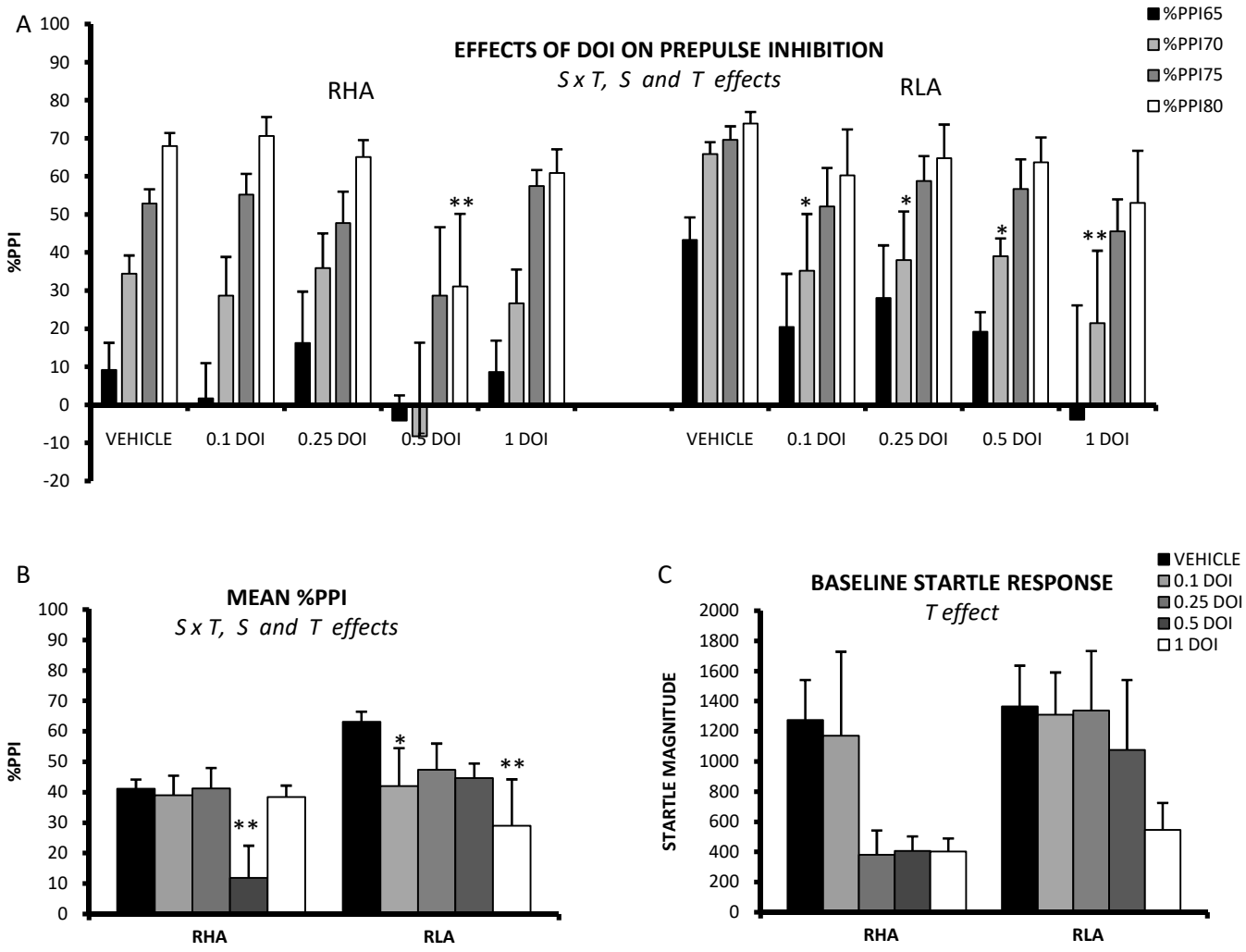

A
EFFECTS OF DOI ON PREPULSE INHIBITION
S x T, S and T effects
RHA
RLA
%PPI
%PPI65
%PPI70
%PPI75
%PPI80
VEHICLE
0.1 DOI
0.25 DOI
0.5 DOI
1 DOI
B
MEAN %PPI
S x T, S and T effects
RHA
RLA
C
BASELINE STARTLE RESPONSE
T effect
STARTLE MAGNITUDE
VEHICLE
0.1 DOI
0.25 DOI
0.5 DOI
1 DOI

Figure 6

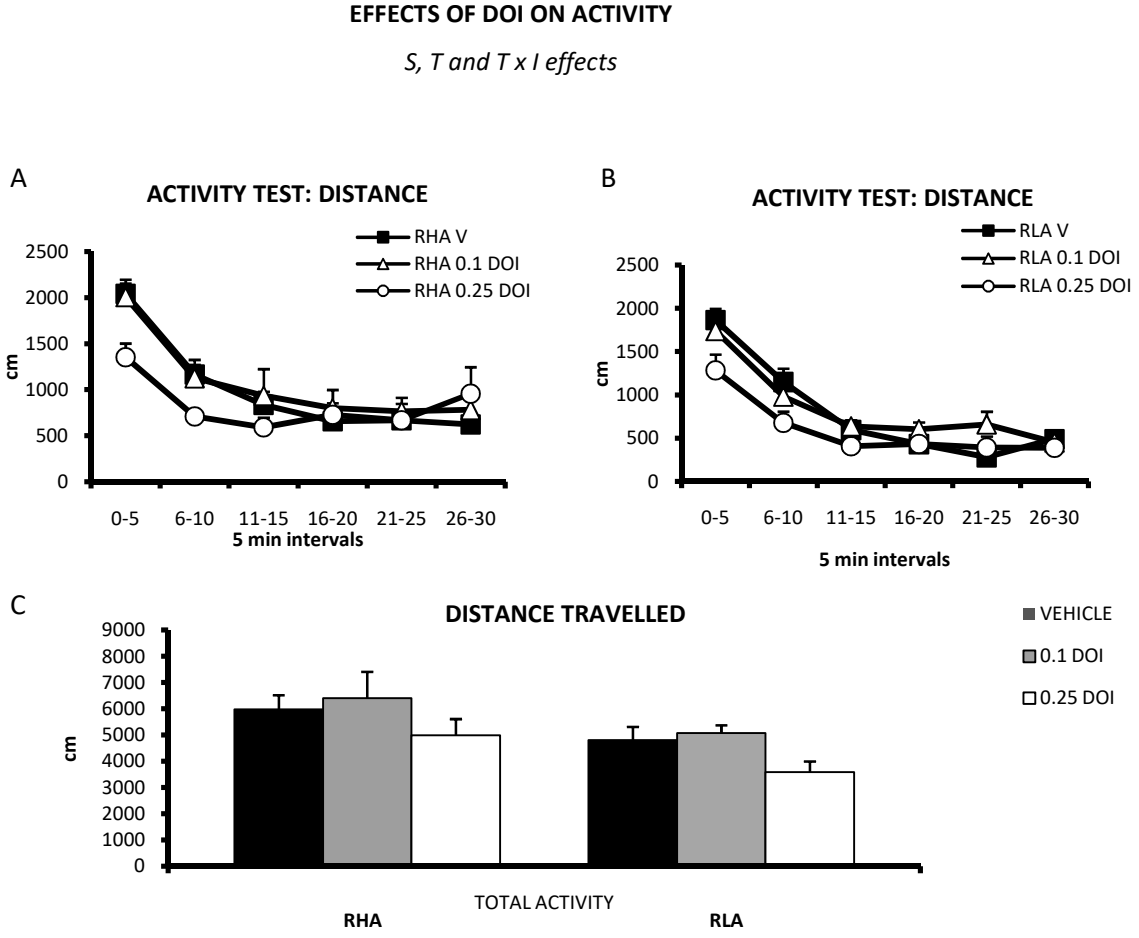

EFFECTS OF DOI ON ACTIVITY
S, T and T x I effects
A
ACTIVITY TEST: DISTANCE
RHA V
RHA 0.1 DOI
RHA 0.25 DOI
cm
2500
2000
1500
1000
500
0
0-5
6-10
11-15
16-20
21-25
26-30
5 min intervals
B
ACTIVITY TEST: DISTANCE
RLA V
RLA 0.1 DOI
RLA 0.25 DOI
cm
2500
2000
1500
1000
500
0
0-5
6-10
11-15
16-20
21-25
26-30
5 min intervals
C
DISTANCE TRAVELLED
VEHICLE
0.1 DOI
0.25 DOI
cm
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
TOTAL ACTIVITY
RHA
RLA

Figure 7

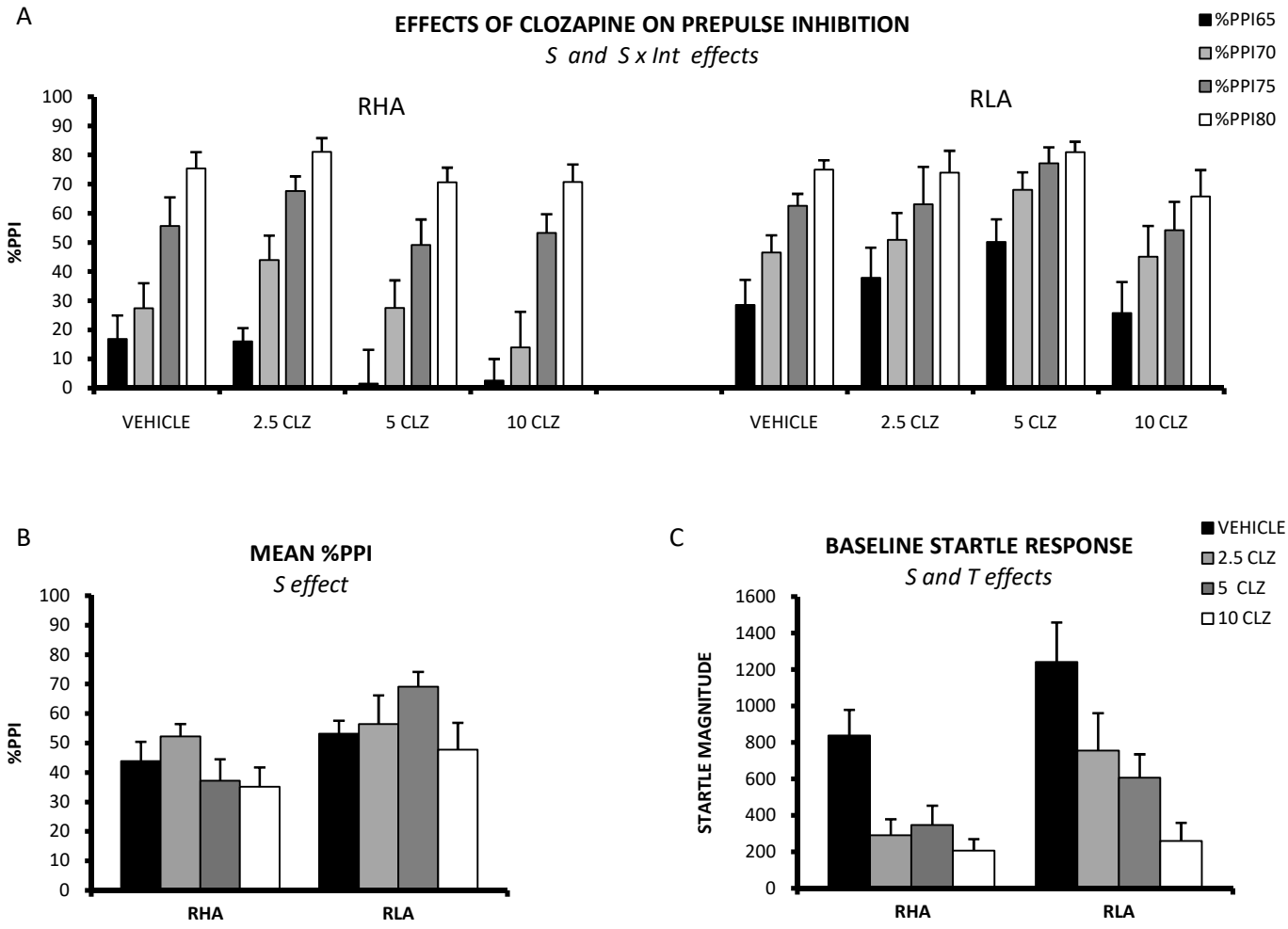

A
EFFECTS OF CLOZAPINE ON PREPULSE INHIBITION
S and S x Int effects
%PPI65
%PPI70
%PPI75
%PPI80
RHA
RLA
%PPI
100
90
80
70
60
50
40
30
20
10
0
VEHICLE
2.5 CLZ
5 CLZ
10 CLZ
VEHICLE
2.5 CLZ
5 CLZ
10 CLZ
B
MEAN %PPI
S effect
%PPI
100
90
80
70
60
50
40
30
20
10
0
RHA
RLA
C
BASELINE STARTLE RESPONSE
S and T effects
VEHICLE
2.5 CLZ
5 CLZ
10 CLZ
STARTLE MAGNITUDE
1600
1400
1200
1000
800
600
400
200
0
RHA
RLA

Figure 8

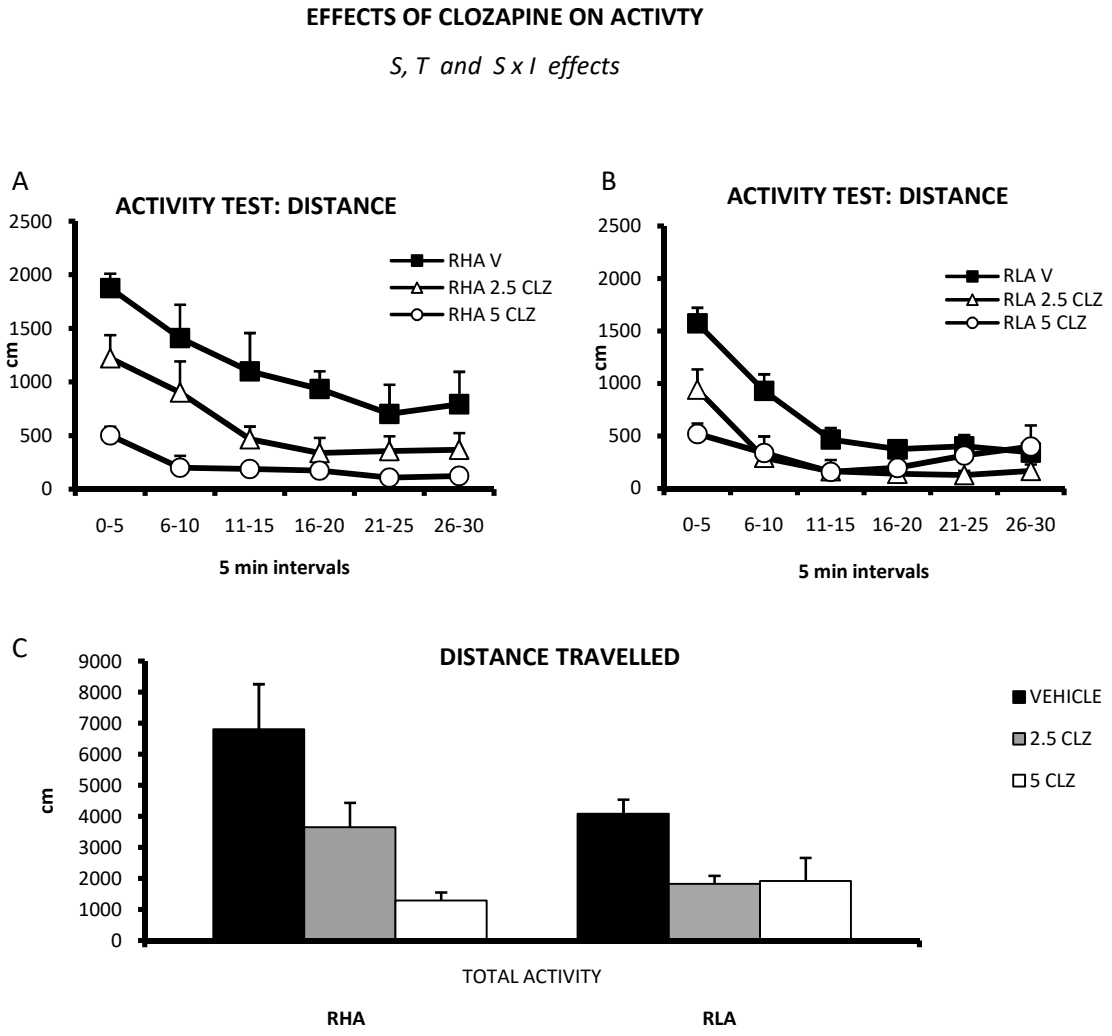

EFFECTS OF CLOZAPINE ON ACTIVTY
S, T and S x I effects
A
ACTIVITY TEST: DISTANCE
RHA V
RHA 2.5 CLZ
RHA 5 CLZ
cm
0-5
6-10
11-15
16-20
21-25
26-30
5 min intervals
B
ACTIVITY TEST: DISTANCE
RLA V
RLA 2.5 CLZ
RLA 5 CLZ
cm
5 min intervals
C
DISTANCE TRAVELLED
VEHICLE
2.5 CLZ
5 CLZ
cm
TOTAL ACTIVITY
RHA
RLA

Figure 9

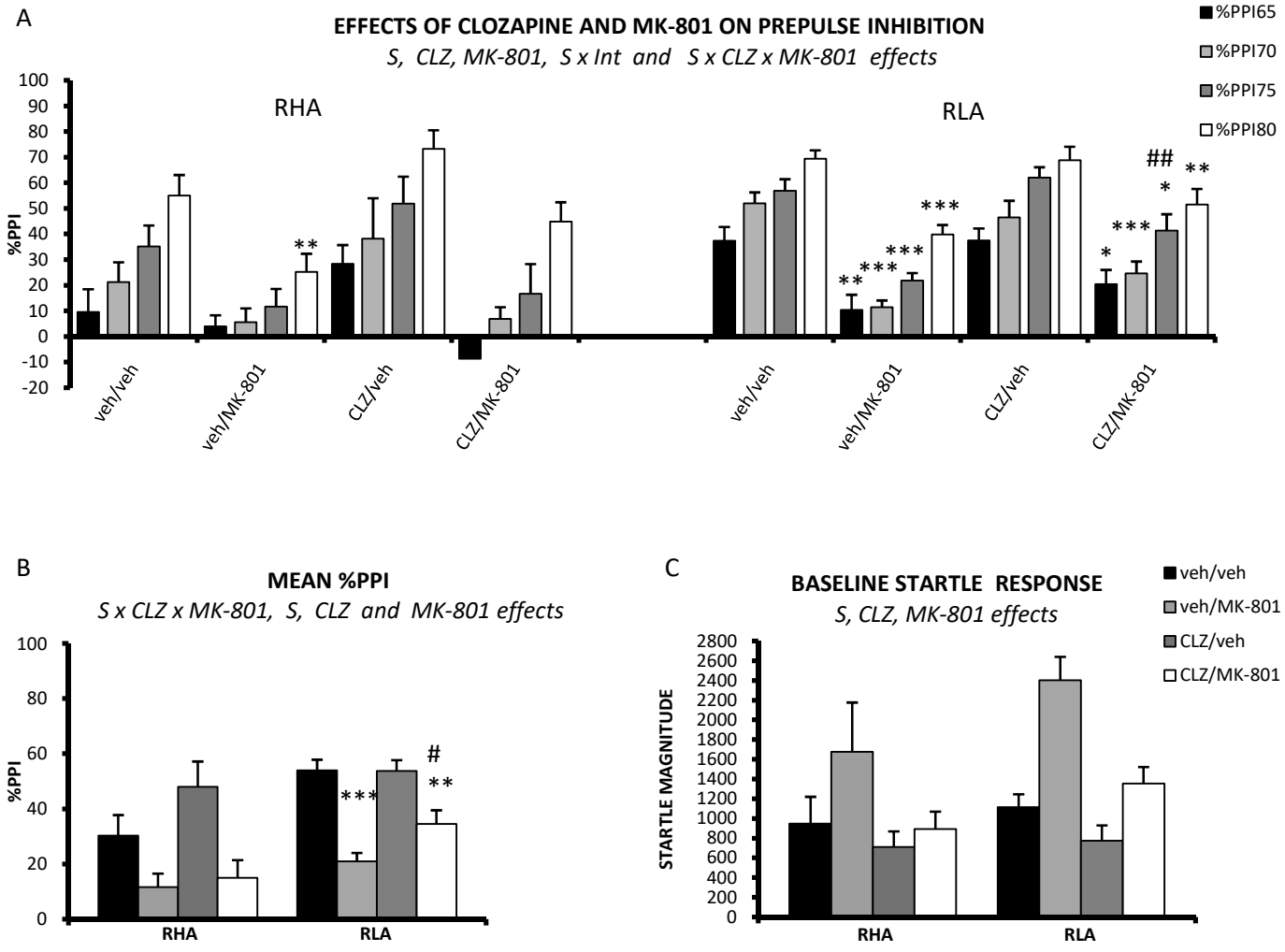

A
EFFECTS OF CLOZAPINE AND MK-801 ON PREPULSE INHIBITION
S, CLZ, MK-801, S x Int and S x CLZ x MK-801 effects
%PPI65
%PPI70
%PPI75
%PPI80
RHA
RLA
%PPI
veh/veh
veh/MK-801
CLZ/veh
CLZ/MK-801
B
MEAN %PPI
S x CLZ x MK-801, S, CLZ and MK-801 effects
RHA
RLA
C
BASELINE STARTLE RESPONSE
S, CLZ, MK-801 effects
STARTLE MAGNITUDE
veh/veh
veh/MK-801
CLZ/veh
CLZ/MK-801

Figure 10

EFFECTS OF CLOZAPINE AND MK-801 ON ACTIVITY

CLZ, MK-801 and CLZ x I effects (30')

CLZ, MK-801, S x CLZ x I and CLZ x I effects (15')

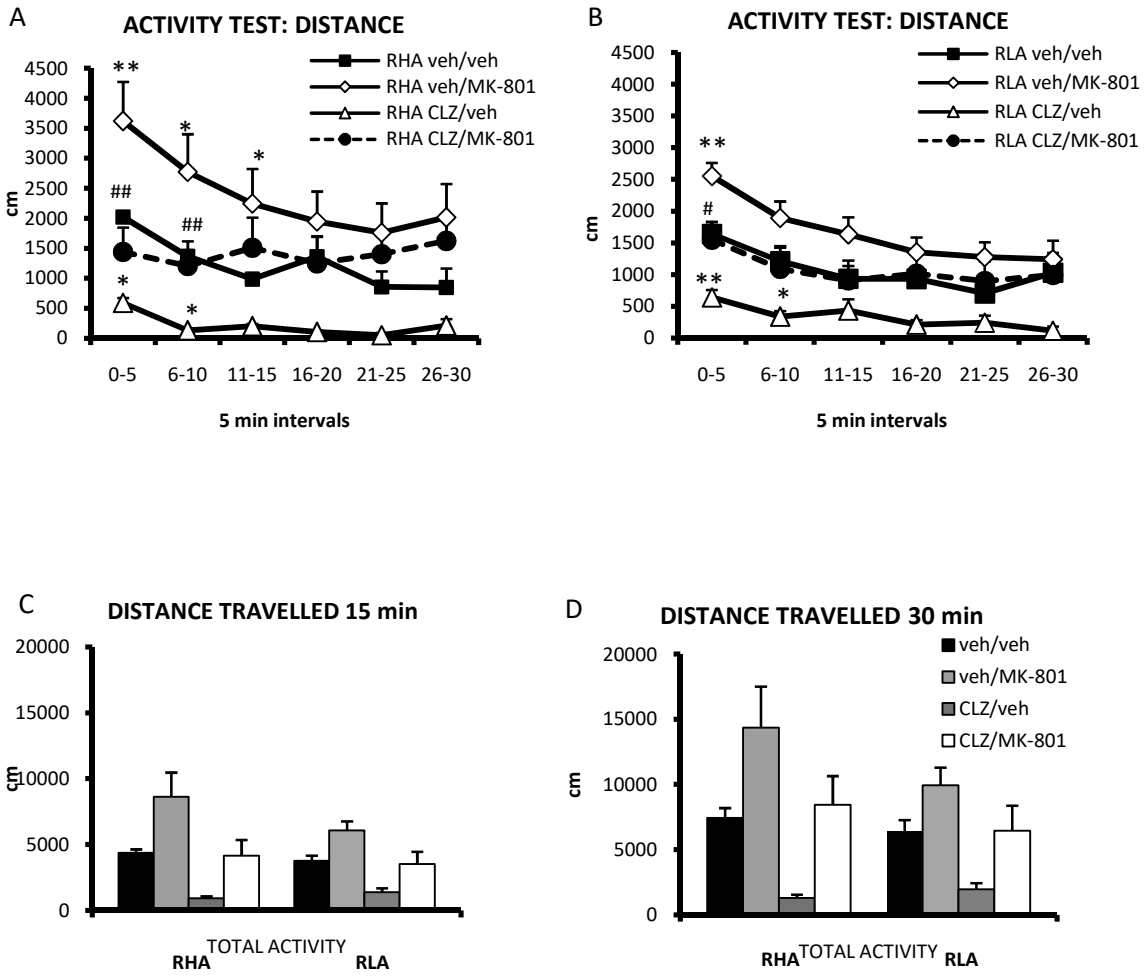


Table 1- Qualitative summary of the main effects on %PPI and locomotor activity observed with the tested drugs.

	RHA		RLA	
	%PPI	Locomotor activity	%PPI	Locomotor activity
Exp. 1, 2-3				
Haloperidol	↑	↓	=	↓↓
Exp. 7-8				
Clozapine	=	↓	=	↓
Exp. 4				
Apomorphine	↓	NT	=	NT
Exp. 5-6				
DOI	↓	↓	↓↓	↓
Exp. 9-10 *(clozapine +MK801)*				
Clozapine	=	↓	=	↓
MK-801	↓	↑↑	↓↓	↑
Clozapine + MK-801 (antagonism of MK-801 effects by CLZ)*	=	↑↑	↑	↑

Symbols: =, no effect; ↑, ↑↑, increase (*, antagonist effect); ↓, ↓↓, decrease or impairment; NT, not tested. CLZ; clozapine.

III. Discussió General

12. Estudi 1

Amb l'objectiu general de caracteritzar les rates Romanes (RHA-I i RLA-I) i les rates genèticament heterogènies (NIH-HS) respecte dels símptomes relacionats amb l'esquizofrènia, com ara els dèficits de PPI i els de memòria de treball, es van realitzar els experiments del l'Estudi 1. Els resultats mostren que les rates RHA-I presenten dèficits de PPI respecte de les RLA-I i les NIH-HS. La diferència entre les soques RHA-I i RLA-I en PPI replica la observada per Del Río et al. (2014), però l'Estudi 1 aporta, a més a més, la nova troballa que les rates genèticament heterogènies NIH-HS presenten nivells de PPI (és a dir, de filtratge atencional) absolutament semblants als de les rates RLA-I. Al test que mesurava memòria de treball (tasca d'aparellament retardat a una posició), en el que les rates han de trobar una plataforma submergida al laberint aquàtic de Morris (en dos assaigs separats per 30 s), els resultats indiquen que les rates RHA-I presenten valors més baixos de memòria de treball que les altres dues soques. També vam veure que les rates RHA-I tenien valors més alts que els altres dos grups en la variable que reflecteix la natació a la perifèria de la piscina (MWM), que indica que les RHA-I segueixen estratègies de natació/orientació desfavorables, ja que la plataforma no es trobava a la perifèria.

També respecte a la memòria de treball, de la mateixa manera del que s'ha observat en la PPI, les rates NIH-HS mostren una execució relativament bona, i indistingible dels valors de la soca RLA-I. Aquesta és la primera caracterització publicada de l'execució de les rates NIH-HS en la PPI i la memòria de treball espacial. Finalment, a la *cued task* ("aprenentatge guiat" per senyals proximals) no vam observar diferències entre soques, fet que ens indica que les rates no tenien cap tipus de problema físic, sensorial o motivacional gruixut que els impedís nadar adequadament.

En la segona part de l'Estudi 1 es van seleccionar, d'una mostra de n=78 rates NIH-HS, les que estaven per sobre/sota d'una desviació estàndard de la mitjana de %PPI, i un grup de rates amb valors intermedis de PPI. Aquests tres grups de rates també van realitzar la tasca de memòria de treball, i els resultats van mostrar que el grup que presentava nivells inferiors de %PPI també tenia valors significativament inferiors en les mesures de memòria de treball respecte dels altres dos grups. A la tasca d'aprenentatge guiat (*cued*

task) no es van observar diferències entre els tres grups, de manera que les diferències en memòria de treball no es poden atribuir a problemes físics, sensorials o motivacionals.

Finalment, amb les dades obtingudes de les rates NIH-HS, es va dur a terme un estudi associacional entre les mesures de PPI i les de memòria de treball, utilitzant correlacions de *Pearson*, anàlisi de regressió múltiple i anàlisi factorial (amb rotació obliqua). Les tres anàlisis van ser coincidents en indicar que hi ha una associació positiva molt consistent entre els nivells de PPI i la memòria de treball, fins al punt que els valors de diverses mesures de la PPI prediuen la bona o mala execució en la tasca de memòria de treball (com indiquen les anàlisi de regressió múltiple; vegeu Estudi 1, *"Table 5"*). Per la seva banda, a l'anàlisi factorial també es veu com les mesures de PPI i les de memòria de treball tenen pesos més alts en el primer factor, cosa que indica que comparteixen variabilitat (vegeu Estudi 1, *"Table 6 B"*).

Aquests estudis de tipus associatiu es van portar a terme amb les rates NIH-HS, que com s'ha descrit a la introducció presenten característiques semblants al que podria ser la població general, sobretot pel que fa a la seva variabilitat genètica, i això fa que els resultats obtinguts amb aquest stock de rates puguin tenir un valor de "translació" i generalització (als humans) superior a si s'utilitzen altres soques de rates estàndard de laboratori. Hi ha diversos estudis que han correlacionat mesures de PPI i altres dominis cognitius en humans (p.ex. Bitsios i Giakoumaki, 2005; Hagan i Jones, 2005; Bitsios et al., 2006; Giakoumaki et al., 2006; Csomor et al., 2009; Young et al., 2009). Tot i això, la possibilitat que la PPI pugui predir les habilitats cognitives, i/o el pronòstic de les persones amb trastorns mentals on el dèficit de PPI hi és present, sembla encara dubtosa (Young et al., 2009; Singer et al., 2013). No obstant, hi ha estudis prometedors, com el de Csomor i col·laboradors (2008), en que es va mostrar una relació entre la PPI i la memòria de treball en persones sanes que es diferenciaven per la seva millor o pitjor execució en la PPI. Per tant, la PPI podria ser una bona eina per tal d'intentar ajudar a predir el pronòstic dels pacients, ja que és una variable fàcil de mesurar i que podria aportar dades significatives de l'estat cognitiu dels pacients (Vargas et al. 2016)

Tenint en compte aquests resultats, que indiquen que la PPI i la memòria de treball comparteixen trets comuns, es pot suggerir que podrien compartir també algunes àrees i/o circuits cerebrals que els regulin. Les rates NIH-HS poden ser un bona eina per diferenciar mecanismes neurals que intervinguin en les funcions representades per la PPI i

la memòria de treball. Una àrea de la que hi ha molta evidència de la seva implicació en aquests dos processos és l'hipocamp (Kohl et al., 2013 Morris et al., 1986a,b; Sawa i Snyder, 2002; Whishaw, 1985; Wible, 2013). En relació amb això, Río-Álamos et al. (2017) ha descrit que les rates RHA-I tenen hipocamps significativament més petits que els de les RLA-I i que el volum de l'hipocamp està positivament correlacionat amb l'execució a la PPI. A més a més, Meyza et al., (2009) i García-Falgueras et al., (2012) han mostrat que les rates RHA-I tenen hipocamps menys funcionals i amb una densitat neuronal menor (respectivament) que les RLA-I. Aquestes diferències hipocampals són per tant consistents amb el fet que les rates RHA-I mostren empitjoraments de la PPI i de la memòria de treball. Reforçant encara més la relació entre hipocamp i PPI, resultats no publicats del nostre laboratori indiquen que les rates heterogènies NIH-HS seleccionades per presentar baixa PPI mostren hipocamps menys voluminosos que les rates NIH-HS seleccionades per alts valors de PPI (Carles Tapias, resultats no publicats).

Aquests resultats, i els estudis de neuroimatge descrits a la "Introducció", indicatius de reducció de l'hipocamp i altres estructures i engrandiment dels ventricles en les rates RHA-I (Río-Álamos, Tesi doctoral en curs), fan pensar en una certa consistència del model animal amb la proposta que les àrees temporals podrien ser molt rellevants per explicar els dèficits cognitius presents en l'esquizofrènia (Goghari et al., 2010; Meyer-Lindenberg, 2010; Millan et al. 2016). Això sembla indicar, que els tractaments enfocats a revertir aquests dèficits atencionals i cognitius haurien de dirigir-se en part a millorar i/o protegir els circuits neuronals de les àrees temporals del cervell. Per exemple, estudis del nostre laboratori mostren que un tipus d'estimulació neonatal (l'anomenat "*neonatal handling"* (NH)) produeix un augment significatiu de la PPI de les RHA-I i de la memòria de treball (Río-Álamos et al. no publicat, Tesi Doctoral en curs). Associat a aquesta millora conductual, també s'observa una lleugera tendència a l'engrandiment del volum de l'hipocamp de les rates RHA-I (Río-Álamos et al. 2017). Tot hi que semblen anar en el sentit adequat (o esperat), aquests resultats, no obstant, no es poden considerar del tot definitius, i esperen replicació.

13. Estudi 2

Al segon estudi es van valorar els efectes conductuals que té l'aïllament social (AS) en les rates Romanes. En primer lloc, en comparació amb les RLA-I, cal dir que, novament, es veu que les rates RHA-I presenten un dèficit de PPI (vegeu també "Estudi 1" d'aquesta Tesi; Del Río et al. 2014), així com menys ansietat (al laberint circular elevat; Rio-Álamos et al. 2015, 2017), més activitat locomotora i un empitjorament de la memòria de treball espacial.

El més important d'aquest estudi és, no obstant, que l'AS va provocar uns dèficits més profunds en les rates RHA-I. Concretament, les rates aïllades d'aquesta soca van mostrar dèficits de PPI (a la intensitat de prepols de 59 dB), hiperactivitat, increments de l'ansietat al laberint circular elevat, i empitjorament de la memòria espacial a llarg termini. En la tasca d'aparellament retardat a una posició (al laberint aquàtic de Morris –MWM-) es va observar que les rates RLA-I aïllades presentaven un dèficit de memòria de treball, respecte a les rates RLA-I no-aïllades, mentre que les rates RHA-I aïllades no van presentar cap dèficit en aquesta tasca. Finalment, en la tasca d'aprenentatge guiat (*cued task*) no es van observar diferències entre els grups, cosa que indica que les diferències obtingudes en les tasques realitzades al MWM no son degudes a problemes físics, motivacionals o sensorials gruixuts.

Per tant, de forma coherent amb la idea que les rates RHA-I serien més vulnerables a la inducció de símptomes "esquizofrènics", aquesta soca presenta una "síndrome d'aïllament" força completa (Fone i Porkess, 2008), que no mostren les rates RLA-I.

El deteriorament de la PPI és un dels dèficits que en la literatura es troben amb més abundància com a conseqüència de l'AS perllongat (Fone i Porkess, 2008). No obstant, a l'Estudi 2 els efectes de l'AS només són significatius a la intensitat més baixa (59 dB). Aquest efecte, menys robust que en d'altres treballs (Bakshi i Geyer, 1999; Domeney i Feldon, 1998, Powell i Geyer, 2002; Powell et al., 2002), pot ser degut a diversos factors, essent un dels més importants la soca d'animals que s'utilitza per estudiar la PPI (Fone i Porkess, 2008; Weiss i Feldon, 2001; per revisió de diferències entre soques vegeu Del Río et al. 2014).

En segon lloc, la hiperactivitat també és un dels fenotips més rellevants i robustos que es troben en la literatura pel que fa al model d'AS (Fone i Porkess, 2008). Els resultats de l'Estudi 2 mostren com les rates RHA-I presenten un augment significatiu de l'activitat locomotora, que és un dels fenotips que es relaciona amb els símptomes positius de l'esquizofrènia (Fone i Porkess, 2008).

En tercer lloc, l'augment de les conductes ansioses que provoca l'AS es veu clarament, ja que els nivells que obtenen les rates RHA-I aïllades en les diferents mesures del laberint circular elevat són iguals als que obtenen les rates RLA-I. És a dir, l'AS crònic hauria incrementat els nivells d'ansietat de les rates RHA-I fins a valors semblants als de les RLA-I (control), d'aquesta manera, les diferències típiques entre les soques s'anul·len en aquest test (p. ex. Díaz-Morán et al. 2012; López-Aumatell et al. 2009b; Martínez-Membrives et al. 2015; Río-Álamos et al. 2015). Cal fer notar que, els resultats produïts per l'AS en aquest test d'ansietat són, en magnitud, els efectes més potents observats en l'Estudi 2 (comparant-los amb els observats en les altres tasques o tests), i són clarament indicatius de conseqüències conductuals molt marcades de l'AS.

Finalment, en les tasques realitzades al MWM les rates RHA-I aïllades mostren dèficits de memòria espacial a llarg termini. Per la seva banda, les rates RLA-I aïllades mostren dèficits de memòria de treball. La inducció dels dèficits cognitius (semblants als presents en l'esquizofrènia) mitjançant l'AS també sembla estar relacionada amb els canvis que produeixen al hipocamp (Muchimapura et al., 2003) i, com s'ha comentat a dalt, les rates RHA-I tenen un hipocamp més vulnerable. Potser com a conseqüència d'això, les RHA-I presenten dèficits cognitius de base i també són més vulnerables als efectes cognitius provocats per l'AS. De tota manera, al marge de la possible (i versemblant) implicació de l'hipocamp, alguns estudis proposen que l'AS afectaria circuits cortico-estriatals i això redundaria en els empitjoraments d'alguns processos d'aprenentatge/memòria espacials tal com es mesuren al MWM (Muchimapura et al., 2003; Wible et al., 2001).

En conjunt, els resultats de l'Estudi 2 donen suport a les rates RHA-I com a model vàlid per estudiar la neurobiologia de l'esquizofrènia, a més a més d'aportar evidències que l'AS en les rates RHA-I provoca l'aparició de conductes o (endo)fenotips relacionats amb l'esquizofrènia (Fone i Porkess, 2008).

14. Estudi 3

El tercer estudi, mostra la caracterització farmacològica, amb fàrmacs antipsicòtics i propsicòtics, de les rates RHA-I (vs. les RLA-I) com a model d'alguns símptomes rellevants per l'esquizofrènia. Els resultats dels experiments de PPI posen de manifest les diferències entre soques que també es van observar a l'Estudi 1 i a Del Río et al. (2014), és a dir que, globalment, les rates RHA-I presenten un dèficit relatiu (vs. les RLA-I) de PPI en tots els experiments de l'Estudi 3.

Respecte als efectes farmacològics de substàncies dopaminèrgiques, l'haloperidol (antagonista dels receptors D2) millora la PPI en les rates RHA-I, mentre que l'apomorfina (agonista mixt dels receptors D1 i D2) redueix la PPI en la mateixa soca de rates. El DOI (agonista preferent dels receptors 5-HT2A) i l'MK-801 (antagonista dels receptors NMDA glutamatèrgics) donen lloc a un empitjorament del filtratge atencional (PPI) de les RLA-I, que és de major magnitud que en les rates RHA-I. La clozapina (antagonista dels receptors 5-HT2A, i d'altres) no modifica els nivells de PPI quan s'administra sola, però si que és capaç de revertir els dèficits de PPI induïts per l'MK-801, tot i que només en les rates RLA-I.

Als experiments on s'avaluava l'activitat locomotora vam observar que les rates RLA-I són més sensibles als efectes reductors de l'activitat de l'haloperidol, i que la clozapina revertia la hiperactivitat induïda per l'MK-801 en les rates RHA-I. Els resultats també mostren que no hi ha efectes diferencials del DOI i de la clozapina entre les dues soques de rates.

Els resultats de l'haloperidol i l'apomorfina són coherents amb els estudis previs en que s'ha vist un to dopaminèrgic (o, funció basal dopaminèrgica) superior de les rates RHA-I, com a conseqüència probable d'una disponibilitat menor (respecte de les RLA-I) d'auto-receptors inhibitoris D2 als cossos cel·lulars de les neurones de les vies nigro-estriada i mesolimbica (p. ex. Guitart-Masip et al., 2008a; Tournier et al., 2013; per revisió vegeu Giorgi et al., 2007, i Driscoll et al., 2009). És a dir, el to dopaminèrgic superior en les rates RHA-I feia esperar el que hem vist, que l'haloperidol (antagonista D2) millorés la PPI més en aquesta soca que en les rates RLA-I, i que l'apormorfina (agonista D1/D2) produís un empitjorament de la PPI també més marcat en la soca RHA-I. Aquests resultats corroboren les hipòtesis inicials, que es basaven sobretot en les diferències

dopaminèrgiques descrites entre ambdues soques (p. ex. Corda et al. 2005; Driscoll et al. 2009; Giorgi et al. 2007; Gimenez-Llort et al. 2005; Guitart-Masip et al. 2006, 2008a-b), i més recentment en el treball de Tournier et al. (2013) respecte a l'auto-receptor D2 (RHA < RLA) i al to dopaminèrgic basal (RHA > RLA), ja que el receptor D2 és la diana molecular de l'haloperidol i de l'apomorfina.

Basant-nos en estudis previs de Sanna et al. (2014a-b) també esperàvem que les rates RLA-I fossin més sensibles als efectes locomotors de l'haloperidol, doncs aquests autors han mostrat que aquest fàrmac redueix la conducta copulatòria de les RLA-I (i no de les RHA-I) probablement per un efecte sobre la seva capacitat locomotora. Això estaria en línia amb els nostres resultats, indicatius que l'haloperidol produeix una major reducció de l'activitat en les rates RLA-I.

La hipòtesi que teníem respecte dels efectes del DOI sobre la PPI es basava, en part, en el treball de Klein et al. (2014), en el que les rates RHA-I presentaven un dèficit dramàtic del receptor mGlu2 de glutamat al CPF, hipocamp i l'estriat, que és per culpa d'un *codó "stop"* prematur provocat per una mutació al gen d'aquest receptor (Wood et al. 2017). També, relacionat amb les evidències anteriors, teníem en compte la demostració del grup de González-Maeso i col·laboradors que els receptors 5-HT2A i mGlu2 formen un complex funcional/fisiològic (anomenat 2AR-mGluR2) que és imprescindible perquè es donin els efectes dels agonistes (al·lucinògens, p.ex. DOI, LSD) i antagonistes 5-HT2A (p. ex. clozapina) (p. ex. Ellaithy et al. 2016; Fribourg et al., 2011; González-Maeso et al. 2007, 2008). Tot plegat suggeria que l'efecte empitjorador del DOI (agonista 5-HT2A) sobre la PPI hauria de ser menys clar a les rates RHA-I, donat que el seu sistema 2AR-mGluR2 està alterat. Els resultats presents donen suport als arguments anteriors, doncs només una dosi de DOI empitjora la PPI en les rates RHA-I, mentre que l'efecte de deteriorament segueix una corba "dosi-efecte" en les RLA-I.

En línia amb els arguments anteriors anirien també els resultats de la clozapina, en especial la reversió parcial dels dèficits de PPI induïts per l' MK-801 en les rates RLA-I. Aquest resultat va en el mateix sentit de diferents treballs que han mostrat que els antipsicòtics atípics reverteixen els efectes dels antagonistes del receptor NMDA sobre la PPI (p. ex. Bubeníková et al., 2005; Fijal et al., 2014; Levin et al., 2005), mentre que l'absència d'antagonisme per la clozapina en les rates RHA-I estaria mitjançada per

l'alteració en el complex 2AR-mGluR2 esmentada a dalt (Klein et al. 2014; González-Maeso et al. 2008).

En el cas de l'activitat motora els resultats mostren el patró contrari, és a dir, la reversió per clozapina de la hiperactivitat induïda per MK-801es produeix en les rates RHA-I. Això pot semblar paradoxal, especialment si pensem que les rates RHA-I són les que tenen un complex 2AR-mGluR2 alterat, sobre el qual la clozapina no podria actuar normalment per modificar la hiperactivitat (induïda per MK-801). La paradoxa, el canvi de sentit dels resultats respecte dels observats en la PPI, pot ser a causa de que la clozapina actua en molts altres receptors (dopaminèrgics, adrenèrgics, histaminèrgics, etc.) (Meltzer et al., 1997; Stahl, 2000), a part dels 5-HT2A, que poden modular l'activitat motora de forma diferencial en les soques romanes. Complementàriament, la participació del receptor 5-HT2A en aquest fenotip (activitat motora) podria ser relativament més petita que en altres fenotips, com ara la PPI. El fet que el DOI no tingui efectes diferencials (entre les soques) sobre l'activitat motora (vegeu Exp. 6 de l' "Estudi 3") podria reforçar aquesta idea que el receptor 5-HT2A (i el complex 2AR-mGluR2) no tingui un paper primordial en l'efecte de la clozapina sobre la hiperactivitat induïda per MK-801. En aquest sentit, respecte del paper dels receptors serotoninèrgics en l'activitat locomotora en rates, Halberstadt et al. (2009, 2012) han proposat que el receptor 5-HT2C té més importància que el 5-HT2A en aquest fenotip (Halberstadt et al., 2009, 2012). Per altra banda, l'antagonisme per part de la clozapina de la hiperactivitat induïda per MK-801 podria estar relacionat amb el fet que l'MK-801 provoca els seus efectes en part per la seva acció sobre vies dopaminèrgiques (Del Arco et al. 2007; Del Arco i Mora 2008, 2009; Jentsch et al. 1998; Meltzer et al. 2011) i, en aquest sentit, el major to dopaminèrgic de la via mesolímbica de les rates RHA-I podria estar relacionat amb els efectes diferencials trobats en aquest experiment (Tournier et al. 2013).

En conjunt, els resultats dels diferents experiments que conformen l'Estudi 3 recolzen la idea que les rates RHA-I poden ser un model animal de símptomes (o processos) lligats a la neurotransmissió dopaminèrgica i/o al complex 2AR-mGluR2, i rellevants per l'esquizofrènia.

15. Estudi 4

A l'estudi d'expressió gènica dels receptors de serotonina 1A i 2A mitjançant la RT-qPCR, els resultats mostren que en les rates RHA-I hi ha un augment de l'expressió del gen del receptor de serotonina 2A (HTR2A) al còrtex prefrontal (CPF) i a l'estriat, mentre que al CPF hi ha un augment de l'expressió del gen del receptor de serotonina 1A (HTR1A), en tots els casos respecte a les rates RLA-I.

Per la seva banda, en les rates NIH-HS dividides en dos grups extrems en nivells de PPI, s'observa que el grup amb menys PPI mostra una certa correspondència amb les rates RHA-I, sobretot pel que fa a l'expressió del HTR1A al CPF. Així, l'expressió gènica d'aquest receptor es troba augmentada al CPF en les rates NIH-HS que van ser seleccionades pels seus valors baixos de PPI, mentre que les rates RHA-I (que com s'ha vist al primer estudi mostren dèficits de PPI) també mostren un augment d'aquest gen respecte de les RLA-I.

Dels estudis associatius, incloent-hi totes les rates dels 4 grups (RHA-I, RLA-I, NIH-HS high-PPI i NIH-HS low-PPI), entre les mesures de PPI i les d'expressió gènica, destaquen algunes correlacions entre les mesures d'expressió gènica dels dos receptors dins de cada àrea i també les correlacions negatives entre algunes variables de la PPI i l'expressió dels receptors al CPF (vegeu *Table 3*, Estudi 4).

Finalment, vam realitzar una anàlisi discriminant lineal en el que les mesures d'expressió del gens HTR1A i HTR2A al CPF s'observa que prediuen correctament la correspondència dels animals als grups de "baixa PPI" (agrupant les RHA-I i les "low-PPI NIH-HS") o "alta PPI" (agrupant les RLA-I i les "high-PPI NIH-HS") en un 87 % dels casos. Per la seva banda, l'expressió de HTR1A al CPF per si sola pot predir la pertinença als grups esmentats d'un 82.6% de casos. Aquests resultats apunten a que l'expressió gènica (o mutacions) dels receptors de serotonina (sobretot el HTR1A) al CPF podrien ser un bon biomarcador per detectar població en risc de patir trastorns mentals caracteritzats pels dèficits de filtratge atencional (Lambe et al., 2011).

Els resultats del gen HTR1A mostren una certa validesa de constructe donat que en estudis amb humans s'ha observat que el receptor 5-HT1A està augmentat en pacients esquizofrènics (Lambe et al., 2011). Per la seva banda, els resultats en humans respecte al receptor 5-HT2A no són tan clars ja que n'hi ha que mostren una disminució mentre que

d'altres no mostren canvis (Baou et al., 2016, Muguruza et al., 2013). No obstant, si es tenen en compte els estudis amb pacients no tractats (és a dir, que no han rebut un tractament crònic amb antipsicòtics atípics), si que s'ha mostrat que en aquests pacients hi ha un augment dels nivells de *binding* del receptor 5-HT2A al CPF (González-Maeso et al., 2008; Muguruza et al., 2013).

Tot i que el present estudi (Estudi 4) mereix una replicació amb una "n" més àmplia, l` idea que en submostres de rates que espontàniament presenten dèficits de PPI es co-seleccionin alteracions sistemàtiques (augments) d'expressió i/o densitat de receptors 5-HT1A i 5-HT2A és molt prometedora, doncs permetria disposar d'un model vàlid per l'estudi de relacions entre filtratge atencional (i, potser, d'altres símptomes relacionats amb l'esquizofrènia) i aspectes de la transmissió serotoninèrgica (5-HT1A, 5-HT2A) i d'altres (com ara la glutamatèrgica; mGlu2R, etc.).

16. Validesa de les rates RHA-I com a model de símptomes relacionats amb l'esquizofrènia

El treball realitzat en la present Tesi parteix dels antecedents resumits a la taula 2 (modificada de Del Río et al., 2014), en la que es mostren totes les característiques per les quals les rates RHA-I poden ser un model vàlid d'alguns símptomes relacionats amb l'esquizofrènia. A partir d'aquests antecedents, a l'Estudi 1 mostrem que les rates RHA-I presenten dèficits de PPI i de memòria de treball, dèficits que formen part dels símptomes cognitius de l'esquizofrènia (Tandon et al., 2013). Per tant, aquest estudi va servir per augmentar la validesa aparent de les rates RHA-I. De la mateixa manera, l'Estudi 2, en el que l'AS va provocar un augment dels símptomes relacionats amb l'esquizofrènia en les rates RHA-I (com ara dèficits de PPI i de memòria a llarg termini, augment de l'ansietat i de l'activitat locomotora), proporciona validesa aparent i de constructe al model, ja que els resultats mostren que aquesta soca de rates és més vulnerable a factors estressants (com l'aïllament) que afecten al neurodesenvolupament.

Respecte a la validesa predictiva, l'Estudi 3, que mostra efectes més marcats de l'haloperidol i l'apomorfina en les rates RHA-I, aporta evidències indirectes consistents amb la idea que hi ha un augment de funció dopaminèrgica al sistema límbic d'aquesta soca de rates. Els resultats del DOI i la "clozapina + MK-801" sobre la PPI, evidencien

que les rates RHA-I són menys sensibles a aquests tractaments, també donen suport indirecte a la idea que els receptors 5-HT2A i mGlu2 són importants pel filtratge atencional (González-Maeso et al., 2007; Wischhof et al., 2012), i que les alteracions observades en aquests receptors en les rates RHA-I poden tenir un paper en els dèficits sensorimotors (Klein et al. 2014; Wood et al. 2017). En resum, els resultats de l'haloperidol, apomorfina, DOI i "clozapina + MK-801" en la PPI, i també en part en l'activitat motora, són congruents amb els resultats obtinguts en diferents estudis en que s'han avaluat les característiques neurobiològiques i els perfils conductuals i psicofarmacològics de les línies de rates romanes (p. ex. Eilam i Szechtman, 1989; Giorgi et al. 2007; Klein et al., 2014; Sanna et al., 2014a-b; Tournier et al., 2013; Wood et al., 2017; vegeu discussió de l'Estudi 3 a dalt).

Es pot dir que els estudis farmacològics de la PPI aporten validesa predictiva al model, sobretot pel que fa al sistema dopaminèrgic, vistos els resultats de l'haloperidol i de l'apomorfina sobre la PPI; però també pel que fa al sistema/complex 2AR-mGluR2, que està alterat en les rates RHA-I i, com a conseqüència, dóna lloc a resultats congruents dels fàrmacs amb acció sobre el receptor 5-HT2A.

Els resultats dels experiments en que es valoraven els efectes de la "clozapina+MK-801" i DOI sobre la PPI, ens indiquen que les diferències entre les soques (RHA-I vs RLA-I) en els sistemes de neurotransmissió serotoninèrgica i glutamatèrgica tenen repercussió funcional, que es reflecteixen en la PPI. Per tant, l'estudi en les rates romanes dels efectes farmacològics de diferents substàncies que interactuïn amb receptors d'aquests sistemes neurotransmissors pot ajudar a conèixer millor els mecanismes serotoninèrgics i glutamatèrgics relacionats amb símptomes de l'esquizofrènia i amb l'acció dels fàrmacs antipsicòtics. En concret, seran necessaris més experiments farmacològics amb les rates romanes que tinguin com a diana el complex 2AR-mGluR2, que juga un paper molt important en el filtratge atencional (p. ex. González Maeso et al. 2007; Wischhof et al. 2012).

Finalment, l'Estudi 4 també aporta un cert grau de validesa de constructe al model, perquè les rates RHA-I presenten un augment dels gens HTR1A i HTR2A al CPF de les rates RHA-I, i s'ha vist en diferents estudis que els pacients esquizofrènics tenen un augment del receptor 5-HT1A (Baou et al. 2016) i del 5-HT2A (González-Maeso et al., 2007; Muguruza et al., 2013). A més a més, hi ha estudis d'associació que han mostrat

una relació entre el gen HTR1A i l'esquizofrènia (Kishi et al., 2011). Per tant, globalment podem dir que els resultats són congruents amb les hipòtesis inicials en les que es proposava a les rates RHA-I com a model d'alguns símptomes i aspectes neuroquímics rellevants per a l'esquizofrènia.

Una limitació important que té aquesta Tesi, és el fet de treballar amb soques consanguínies, perquè presenten una variabilitat genètica i fenotípica menor. Això es reflecteix, per exemple, en la mutació, recentment descoberta per Wood i col·laboradors (2017), i que té una penetrància del 100% en les rates RHA-I. No obstant, aquesta limitació s'ha intentat superar incloent la soca de rates heterogènies NIH-HS. Aquesta soca presenta una variabilitat fenotípica i genètica molt més gran. En aquest sentit, tant els resultats conductuals de l'Estudi 1 com en els estudis d'expressió gènica (Estudi 4) mostren que les rates NIH-HS obtenen valors semblants a les rates RLA-I. Per tant, semblaria que les rates RLA-I són un bon control per tal de comparar els resultats de les RHA-I, i això faria augmentar el valor translacional de la majoria de resultats dels experiments realitzats amb les rates romanes d'aquesta Tesi.

Globalment, per tal de superar aquestes limitacions seria important realitzar altres tipus de tests conductuals per valorar la memòria de treball i també altres processos cognitius, per tal d'ampliar la validesa aparent de les rates RHA-I (Estudi 1). També es podria incloure la soca NIH-HS en els estudis amb l'AS i els farmacològics (Estudis 2 i 3), per tal d'augmentar la capacitat d'extrapolar els resultats trobats amb les rates RHA-I.

17. Què aporten les rates RHA-I/RLA-I i les NIH-HS dins dels models animals de l'esquizofrènia o dels seus símptomes?

Les rates genèticament heterogènies (NIH-HS), en contraposició a les rates romanes, presenten unes característiques especials de variabilitat fenotípica i genotípica que les fan més semblants a la població general (p. ex. Johannesson et al. 2009; Baud et al. 2013). Per aquest motiu, a l'Estudi 1, els resultats que mostren que la PPI i la memòria de treball estan correlacionades, que la PPI pot predir l'execució a la tasca de memòria de treball espacial i que els dos processos cognitius comparteixen trets comuns (comparteixen variància a l'anàlisi factorial), prenen més importància encara per haver estat trobats amb les rates NIH-HS, ja que les conclusions que se'n deriven tenen un potencial valor

translacional i de generalització més alt que si s'haguessin trobat només en soques consanguínies de rates (com ara les RHA-I/RLA-I) o en d'altres soques d'ús habitual (p. ex. Sprague Dawley, Wistar, Long Evans, etc; vegeu p.ex. Baud et al., 2013, 2014a-b).

Diferents estudis han posat de manifest que en l'esquizofrènia es donen dèficits de PPI (Greenwood et al., 2016; Roussos et al., 2016), de manera que la PPI s'ha considerat un endofenotip útil per a la recerca neurobiològica sobre aspectes (p. ex. atencionals) lligats a la malaltia. Es considera necessari i important, no obstant, investigar si el filtratge atencional (representat per la PPI, entre d'altres mesures) està relacionat amb altres processos cognitius afectats en l'esquizofrènia, i conèixer amb quines funcions (alterades o afectades) comparteixen elements i amb quines no, doncs això pot guiar la recerca sobre mecanismes neurals compartits o separats i sobre tractaments més dirigits a "*clusters*" particulars de símptomes/processos (p. ex. Singer et al., 2013; Csomor et al., 2008). No obstant això, no ha estat fàcil demostrar la relació de la PPI amb d'altres processos cognitius que també estan afectats en l'esquizofrènia i, tot i alguns resultats positius, l'evidència de relació entre PPI i memòria (com a exemple de procés cognitiu sovint afectat en l'esquizofrènia) encara està en discussió (Bitsios i Giakoumaki, 2005; Bitsios et al., 2006; Csomor et al., 2009; Giakoumaki et al., 2008).

Les observacions de l'Estudi 1 amb les rates NIH-HS (que al seu torn reforcen el que s'observa amb les RHA-I/RLA-I) representen la primera vegada que l'associació entre PPI i memòria de treball és observada en rates genèticament heterogènies. A més a més, l'acord entre les diferents tipus d'anàlisi i amb els resultats de les rates RHA-I vs. RLA-I, donen una especial consistència als resultats, suggerint que en condicions de dèficits en PPI (dèficits sensorimotors o de filtratge atencional), com ara en l'esquizofrènia o en models animals, altres funcions afectades (com la memòria de treball) podrien compartir alguns mecanismes neurals. Posant en valor els arguments anteriors, al nostre laboratori hem observat que les rates RHA-I, que, recordem, mostren dèficits de PPI i memòria de treball, presenten un volum hipocampal inferior al de les RLA-I (Río-Álamos et al. 2017) i, així mateix, les rates NIH-HS amb baixa PPI presenten un volum hipocampal inferior a les NIH-HS amb alta PPI (Carles Tapies-Espinosa, Tesi Doctoral en curs). En estudis en curs en la mateixa línia s'està investigant si l'hipocamp, i d'altres estructures cerebrals implicades en la modulació de la PPI, presenten alteracions en la funcionalitat entre rates NIH-HS estratificades per la seva alta o baixa PPI.

En el mateix context, a l'Estudi 4 hem vist que les rates NIH-HS, dividides pels seus valors extrems de PPI, presenten un patró d'expressió del gen HTR1A similar al que presenten les rates romanes. És a dir, les rates amb nivells baixos de PPI (RHA-I i les low-PPI NIH-HS) , presenten nivells d'expressió del gen al CPF significativament més alts que les rates amb nivells alts de PPI (RLA-I i high-PPI NIH-HS). En estudis amb pacients esquizofrènics s'ha vist que aquest receptor està augmentat al CPF (Lambe et al., 2011). Per tal de confirmar els resultats de les rates NIH-HS de l'Estudi 4 s'està portant a terme l'avaluació d'expressió gènica amb un nombre més gran d'animals també estratificats per valors de PPI alts, baixos o intermedis.

Les rates RHA-I presenten un rang força ampli de trets, tant conductuals com neuroquímics i farmacològics (vegeu Taula 2), que són congruents amb les característiques que haurien de presentar els models animals de l'esquizofrènia respecte al símptomes positius (p. ex. hiperactivitat davant la novetat), símptomes negatius (p. ex. alteracions en la conducta de nidació, agressivitat) i símptomes cognitius (p. ex. dèficits de memòria de treball, dèficits d'atenció sostinguda al 5-CSRTT). Altres models animals que han seleccionat les rates per una característica concreta, també presenten molts fenotips relacionats amb l'esquizofrènia. Per exemple, les rates susceptibles (o no susceptibles) als efectes de l'apomorfina, presenten dèficits de PPI i d'inhibició latent i algunes característiques neuroquímiques relacionades amb la neurotransmissió dopaminèrgica que són consistents amb les que presenten els pacients esquizofrènics (Ellenbroek i Karl, 2016).

De la mateixa manera, la rates Brattleboro, que tenen una mutació que provoca que alliberin menys vasopresina, presenten dèficits de PPI, inhibició latent i d'habituació de la resposta de sobresalt (Ellenbroek i Karl, 2016; Del Río et al. 2014). A part d'això, diferents estudis farmacològics han vist que tant els antipsicòtics típics com els atípics augmenten la PPI d'aquesta soca de rates (Cilia et al., 2010), fet que aporta validesa predictiva a aquest model (Geyer et al., 2001, 2012). No obstant, cal dir que els experiments que han avaluat els efectes dels antipsicòtics actuals sobre la PPI en humans (pacients esquizofrènics) ofereixen resultats encara controvertits (Ellenbroek i Karl, 2016).

Per altra banda, les rates espontàniament hipertenses (*spontaneous hypertensive rats*, SHR) també presenten alguns fenotips com hiperactivitat i dèficits de PPI, d'interacció

social i de por condicionada a un context. Tots aquests dèficits són reversibles administrant antipsicòtics (Ellenbroek i Karl, 2016), el que posa de manifest que aquest model té validesa aparent i predictiva.

Les rates seleccionades i criades pels seus valors baixos de PPI també presenten característiques rellevants, com són les alteracions de la conducta social, reducció de la motivació i augment de la perseveració en diferents tasques. En aquesta soca de rates els dèficits de PPI són revertits per l'haloperidol (Ellenbroek i Karl, 2016; Hadamitzky et al., 2007).

Tenint en compte que l'experimentació amb les rates romanes va començar amb Bignami (1965), la caracterització que s'ha fet de les rates RHA-I, en relació a símptomes o fenotips rellevants per l'esquizofrènia, és força extensa, en comparació amb les altres soques de rates seleccionades esmentades abans. A més a més, les troballes recents de Klein et al. (2014) i Fomsgaard et al. (2017) sobre els mecanismes serotoninèrgics i glutamatèrgics que diferencien les rates romanes, indiquen que les RHA-I presenten certes característiques neuroquímiques que no s'han vist fins ara en altres models genètics de l'esquizofrènia en rates, com són els increments de certs receptors serotoninèrgics i el dèficit de receptors mGlu2 glutamatèrgics. Donada la importància que investigacions recents atribueixen al complex receptorial 2AR-mGluR2 respecte de la simptomatologia esquizofrènica (p. ex. González-Maeso et al., 2008; Liu et al., 2012; Wischhof i Koch, 2016), els aspectes esmentats semblen atorgar un valor especial a les rates RHA-I com a model per a la recerca sobre aquest trastorn.

D'altra banda, les rates RHA-I presenten (com hem dit a dalt) un volum hipocampal menor que les RLA-I, a més a més d'un volum del CPF també disminuït i un engrandiment molt important (de l'ordre del doble de volum que en les RLA-I) dels ventricles laterals (Rio-Álamos et al. sotmès), totes elles característiques que s'han relacionat amb l'esquizofrènia i que, fins on coneixem, no presenten les altres soques de rates esmentades a dalt (p. ex. Ellenbroek i Karl, 2016; Sawa i Snyder 2002).

Les característiques conductuals/farmacològiques, neuroquímiques/moleculars i neuro-estructurals comentades, creiem que fan de les rates RHA-I un model prometedor per la recerca sobre símptomes i processos relacionats amb l'esquizofrènia, doncs presenten algunes característiques rellevants per aquest trastorn que estan absents en els altres models genètics de rata esmentats.

En resum, els estudis d'aquesta Tesi, pel fet d'utilitzar les tres soques de rates (RHA-I, RLA-I i NIH-HS) i que una d'elles és la més heterogènia genèticament que existeix en l'actualitat, presenten possiblement l'avantatge que així s'augmenta la validesa externa (capacitat d'extrapolar els resultats a d'altres contexts) i la translacionalitat dels resultats. Cal destacar també que el fet de comptar amb les rates RLA-I com a soca de comparació és rellevant, doncs, d'una banda, s'han revelat com una soca resistent a desenvolupar símptomes "esquizofrènics" fins i tot després d'un tractament perllongat d'AS (Estudi 2), mentre que, per altra banda, sí que es mostren sensibles als dèficits (en PPI, al menys) induïts per substàncies "propsicòtiques", com ara el DOI o el MK-801 (Estudi 3).

Pel que fa a la continuació de la recerca en la línia marcada per aquesta Tesi, l'estudi sistemàtic de simptomatologia negativa (p. ex. dèficits socials) en les tres soques de rates, i la relació amb la PPI, és una de les línies que s'està desenvolupant al nostre laboratori. Al mateix temps, s'estan portant a terme: 1) estudis d'activació de cFos (per mesurar activitat neuronal en àrees suposadament moduladores de la PPI i d'altres símptomes de l'esquizofrènia), nivells cerebrals de COMT i volumetria cerebral per MRI en les tres soques (Tapias-Espinosa, Tesi en curs); 2) estudis d'expressió gènica del complex 2AR-mGluR2 en relació amb la PPI (Oliveras, treball en curs); 3) avaluació de densitat sinàptica i neurones/sinapsis inhibitòries (GABAèrgiques) fronto-corticals (Sánchez-González, Tesi en curs) en les rates RHA-/RLA-I i, 4) estudis sobre els efectes de tractaments ambientals infantils sobre la PPI, la memòria de treball (i d'altres fenotips) i els volums d'estructures cerebrals rellevants en les rates RHA-I/RLA-I (Río-Álamos, Tesi en curs; Río-Álamos et al. 2017).

De l'abordatge combinat anterior s'espera extreure conclusions sobre la relació entre fenotips conductuals/psicològics rellevants per l'esquizofrènia (com la PPI o d'altres representatius de simptomatologia negativa o cognitiva) i processos neurals implicats en el trastorn o en símptomes típics del mateix.

Taula 2.- *Fenotips conductuals i neurobiològics rellevants per l'esquizofrènia en les rates RHA (de Del Río et al. 2014)*

	Símptomes esperats dels models animals	**RHA vs RLA**
I.- Símptomes positius	A.- Activitat locomotora en resposta a la novetat	*RHA: major activitat locomotora (també comparant-les amb les rates Sprague Dawley).
	B.- Sensibilitat a drogues psicotomimètiques/psicoestimulants:	
	-Augment de la locomoció/estereotípies quan s'administren agonistes DA	*RHA: Augment de les estereotípies quan s'administra apomorfina (també comparant-les amb les rates Sprague-Dawley) i augment de la locomoció quan s'administra amfetamina.
	-Augment locomoció i sensitització als agonistes dopaminèrgics	*RHA: augment de la locomoció i de la sensitització als agonistes dopaminèrgics com les amfetamines (també comparant-les amb les rates Sprague Dawley).
II.- Símptomes negatius	A.- Disminució de conductes d'anidament	*RHA: dèficits de conductes d'anidament.
	B.- Conductes socials: *Resident-Intruder* test	*RHA: Increment en la latència d'agressió.
III.- Símptomes cognitius	A.- Dèficits de memòria de treball	*RHA: Disminució de la memòria de treball en la tasca d'aparellament retardat a una posició MWM (també comparant-les a les rates NIH-HS -Estudi 1 d'aquesta Tesi-).
	B.- Dèficits en filtratge atencional i altres funcions executives:	
	-Dèficits de PPI	*RHA: alteracions en la PPI (vs RLA i també les rates NIH-HS rats -Estudi 1-).
	-Dèficits en inhibició latent:	RHA: Dèficits d'inhibició latent.
	-*5-choice serial reaction time test* (5-CSRTT)	*RHA: Augment de respostes prematures i impulsivitat.
	C.- Altres dèficits cognitius	RHA: Alteracions en tasques d'aprenentatge espacial de referència (i altres tasques de memòria explicita o declarativa) al laberint aquàtic de Morris i al laberint Hebb-Williams. Alteracions en el condicionament clàssic aversiu (també comparant-les amb les rates NIH-HS).
IV.- Fenotips neuroquímics i neuroanatòmics relacionats amb l'esquizofrènia	A.- Neurotransmissió dopaminèrgica	*RHA: Augment de la dopamina de les vies mesolímbica i mesocortical davant d'agonistes dopaminèrgics i d'estrès.
	B.- Neurotransmissió serotoninèrgica i glutamatèrgica	*RHA: expressió dels receptors 5-HT1A 5-HT2A augmentada al còrtex prefrontal (veure Estudi 4 pels gens *HTR1A* i *HTR2A*) i absència d'expressió del mGluR2 al còrtex prefrontal còrtex, hipocamp i estriat. Perfil del complex receptorial 5HT2A/mGluR2 que s'assembla al que tenen els pacients esquizofrènics i al dels ratolins knockout pel receptor mGluR2.
	C.- Morfologia/funció hipocàmpica, còrtex prefrontal (CPF) i ventricles laterals	* RHA: Reducció de funció hipocàmpica de densitat neuronal de les regions CA1-CA3. Disminució de volum del CPF i increment dramàtic de volum de ventricles laterals (fenotips semblants als dels malalts esquizofrènics).

18. Conclusions

1. Les rates de la soca RHA-I presenten dèficits de PPI i de memòria de treball comparant-les amb les rates RLA-I i les heterogènies NIH-HS (Estudi 1).

2. Per primera vegada es demostra en rates que existeix una associació positiva consistent entre la PPI i la memòria espacial de treball (Estudi 1).

3. L'aïllament social crònic va provocar un augment dels fenotips típics del que s'anomena "*isolation syndrome"* ("síndrome d'aïllament") a les rates RHA-I. Així, en aquesta soca (però no en les RLA-I) l'aïllament indueix dèficits de PPI, hiperactivitat, augment de l'ansietat i deteriorament de la memòria espacial a llarg termini (Estudi 2).

4. Les rates RHA-I són més sensibles (que les RLA-I) als efectes milloradors de l'haloperidol i els efectes empitjoradors de l'apomorfina en la PPI, en coherència amb la seva major funcionalitat dopaminèrgica central (Estudi 3).

5. En comparació amb les rates RHA-I, el DOI empitjora més la PPI en la soca RLA-I, i la clozapina reverteix més clarament els dèficits de PPI induïts per l'MK-801 en aquesta soca, el que pot ser a causa de que les rates RHA-I presenten una alteració del complex 2AR-mGluR2 (Estudi 3).

6. Pel que fa a l'activitat locomotora, les rates RHA-I són més sensibles als efectes de la clozapina, ja que va ser capaç de disminuir la hiperactivitat induïda per l'MK-801, mentre que les rates RLA-I són més sensibles als efectes reductors de l'activitat de l'haloperidol. Ambdós efectes podrien també estar relacionats amb les diferències en funció dopaminèrgica entre les dues soques (Estudi 3).

7. Per tant, les diferències en efectes farmacològics (sobre la PPI i l'activitat locomotora) entre les rates RHA-I i RLA-I podrien ser degudes a les diferències entre les soques pel que fa a la neurotransmissió dopaminèrgica i serotoninèrgica, i les marcades diferències respecte al complex format pels receptors 5-HT2A i mGlu2 (Estudi 3).

8. Respecte de les RLA-I, les rates RHA-I (que tenen nivells baixos de PPI) mostren un augment de l'expressió dels gens HTR1A i HT2A al còrtex prefrontal i del HTR2A a l'estriat respecte a les RLA-I, i les rates NIH-HS amb nivells baixos de PPI mostren un augment de l'expressió del gen HTR1A al còrtex prefrontal (Estudi 4).

9. L'estudi correlacional i l'anàlisi discriminant mostren que nivells alts d'expressió del gen HTR1A estan associats als dèficits de PPI, i que l'expressió d'aquest gen és capaç de discriminar els subjectes en risc de mostrar aquests dèficits (Estudi 4).

IV. Bibliografia

Abbott, A., 2010. Schizophrenia: The drug deadlock. Nature 468, 158–159.

Abi-Dargham, A., van de Giessen, E., Slifstein, M., Kegeles, L.S., Laruelle, M., 2009. Baseline and amphetamine-stimulated dopamine activity are related in drug-naïve schizophrenic subjects. Biol. Psychiatry 65, 1091–1093.

Aghajanian, G.K., Marek, G.J., 2000. Serotonin model of schizophrenia: emerging role of glutamate mechanisms. Brain Res. Rev. 31, 302–312.

Aguilar, R., Escorihuela, R.M., Gil, L., Tobeña, A., Fernández-Teruel, A., 2002. Differences between two psychogenetically selected lines of rats in a swimming pool matching-to-place task: Long-term effects of infantile stimulation. Behav. Genet. 32, 127–134.

Alam, I., Koller, D.L., Cañete, T., Blázquez, G., López-Aumatell, R., Martínez-Membrives, E., Díaz-Morán, S., Tobeña, A., Fernández-Teruel, A., Stridh, P., Diez, M., Olsson, T., Johannesson, M., Baud, A., Econs, M.J., Foroud, T., 2014. High-resolution genome screen for bone mineral density in heterogeneous stock rat. J. Bone Miner. Res. 29, 1619–26.

Alam, I., Koller, D.L., Sun, Q., Roeder, R.K., Cañete, T., Blázquez, G., López-Aumatell, R., Martínez-Membrives, E., Vicens-Costa, E., Mont, C., Díaz, S., Tobeña, A., Fernández-Teruel, A., Whitley, A., Strid, P., Diez, M., Johannesson, M., Flint, J., Econs, M.J., Turner, C.H., Foroud, T., 2011. Heterogeneous stock rat: A unique animal model for mapping genes influencing bone fragility. Bone 48, 1169–1177.

American Psychiatric Association, 2013. Diagnostic and statistical manual of mental disorders, APA, Washington, DC, (5th ed.).

Antonova, E., Sharma, T., Morris, R., Kumari, V., 2004. The relationship between brain structure and neurocognition in schizophrenia: a selective review. Schizophr. Res. 70, 117–145.

Asenjo Lobos, C., Komossa, K., Rummel-Kluge, C., Hunger, H., Schmid, F., Schwarz, S., Leucht, S., 2010. Clozapine versus other atypical antipsychotics for schizophrenia. Cochrane database Syst. Rev. 11, CD006633.

Aultman, J.M., Moghaddam, B., 2001. Distinct contributions of glutamate and dopamine receptors to temporal aspects of rodent working memory using a clinically relevant task. Psychopharmacology (Berl). 153, 353–64.

Bakshi, V.P., Geyer, M.A., 1999. Ontogeny of isolation rearing-induced deficits in sensorimotor gating in rats. Physiol. Behav. 67, 385–392.

Baou, M., Boumba, V.A., Petrikis, P., Rallis, G., Vougiouklakis, T., Mavreas, V., 2016. A review of genetic alterations in the serotonin pathway and their correlation with psychotic diseases and response to atypical antipsychotics. Schizophr. Res. 170, 18–29.

Baud, A., Guryev, V., Hummel, O., Johannesson, M., Hermsen, R., Stridh, P., Graham, D., McBride, M.W., Foroud, T., Calderari, S., Diez, M., Ockinger, J., Beyeen, A.D., Gillett, A., Abdelmagid, N., Guerreiro-Cacais, A.O., Jagodic, M., Tuncel, J., Norin, U., Beattie, E., Huynh, N., Miller, W.H., Koller, D.L., Alam, I., Falak, S., Osborne-Pellegrin, M., Martínez-Membrives, E., Cañete, T., Blázquez, G., Vicens-Costa, E., Mont-Cardona, C., Díaz-Morán, S., Tobeña, A., Zelenika, D., Saar, K., Patone, G., Bauerfeind, A., Bihoreau, M.-T., Heinig, M., Lee, Y.-A., Rintisch, C., Schulz, H., Wheeler, D.A., Worley, K.C., Muzny, D.M., Gibbs, R.A., Lathrop, M., Lansu, N., Toonen, P., Ruzius, F.P., de Bruijn, E., Hauser, H., Adams, D.J., Keane, T., Atanur, S.S., Aitman, T.J., Flicek, P., Malinauskas, T., Jones, E.Y., Ekman, D., Lopez-Aumatell, R., Dominiczak, A.F., Holmdahl, R., Olsson, T., Gauguier, D., Hubner, N., Fernández-Teruel, A., Cuppen, E., Mott, R., Flint, J., 2014. Genomes and phenomes of a population of outbred rats and its progenitors. Sci. Data 1,140011.

Baud, A., Hermsen, R., Guryev, V., Stridh, P., Graham, D., McBride, M.W., Foroud, T., Calderari, S., Diez, M., Ockinger, J., Beyeen, A.D., Gillett, A., Abdelmagid, N., Guerreiro-Cacais, A.O., Jagodic, M., Tuncel, J., Norin, U., Beattie, E., Huynh, N., Miller, W.H., Koller, D.L., Alam, I., Falak, S., Osborne-Pellegrin, M., Martínez-Membrives, E., Cañete, T., Blázquez, G., Vicens-Costa, E., Mont-Cardona, C., Díaz-Morán, S., Tobeña, A., Hummel, O., Zelenika, D., Saar, K., Patone, G., Bauerfeind, A., Bihoreau, M.-T.,

Heinig, M., Lee, Y.-A., Rintisch, C., Schulz, H., Wheeler, D.A., Worley, K.C., Muzny, D.M., Gibbs, R.A., Lathrop, M., Lansu, N., Toonen, P., Ruzius, F.P., de Bruijn, E., Hauser, H., Adams, D.J., Keane, T., Atanur, S.S., Aitman, T.J., Flicek, P., Malinauskas, T., Jones, E.Y., Ekman, D., López-Aumatell, R., Dominiczak, A.F., Johannesson, M., Holmdahl, R., Olsson, T., Gauguier, D., Hubner, N., Fernández-Teruel, A., Cuppen, E., Mott, R., Flint, J., 2013. Combined sequence-based and genetic mapping analysis of complex traits in outbred rats. Nat. Genet. 45, 767–775.

Bender, S., Weisbrod, M., Resch, F., 2007. Which perspectives can endophenotypes and biological markers offer in the early recognition of schizophrenia? J. Neural Transm. 114, 1199–1215.

Bignami, G., 1965. Selection for high rates and low rates of avoidance conditioning in the rat. Anim. Behav. 13, 221–227.

Bitsios, P., Giakoumaki, S.G., 2005. Relationship of prepulse inhibition of the startle reflex to attentional and executive mechanisms in man. Int. J. Psychophysiol. 55, 229–41.

Bitsios, P., Giakoumaki, S.G., Theou, K., Frangou, S., 2006. Increased prepulse inhibition of the acoustic startle response is associated with better strategy formation and execution times in healthy males. Neuropsychologia 44, 2494–2499.

Bleuler, E., 1911. Dementia Praecox oder Gruppe der Schizophrenien. Deuticke, Leipzig

Bleuler, E., 1950. Dementia Praecox or the Group of Schizophrenias. International Universities Press, New York. (Zinkin J, translator)

Boksa, P., El-Khodor, B.F., 2003. Birth insult interacts with stress at adulthood to alter dopaminergic function in animal models: possible implications for schizophrenia and other disorders. Neurosci. Biobehav. Rev. 27, 91–101.

Braff, D., Stone, C., Callaway, E., Geyer, M., Glick, I., Bali, L., 1978. Prestimulus effects on human startle reflex in normals and schizophrenics. Psychophysiology 15, 339–343.

Bubeníková, V., Votava, M., Horácek, J., Pálenícek, T., Dockery, C., 2005. The effect of zotepine, risperidone, clozapine and olanzapine on MK-801-disrupted sensorimotor gating. Pharmacol. Biochem. Behav. 80, 591–6.

Buckley, P.F., Miller, B.J., Lehrer, D.S., Castle, D.J., 2009. Psychiatric comorbidities and schizophrenia. Schizophr. Bull. 35, 383–402.

Carrasco, J., Márquez, C., Nadal, R., Tobeña, A., Fernández-Teruel, A., Armario, A., 2008. Characterization of central and peripheral components of the hypothalamus-pituitary-adrenal axis in the inbred Roman rat strains. Psychoneuroendocrinology 33, 437–45.

Cilia, J., Gartlon, J.E., Shilliam, C., Dawson, L.A., Moore, S.H., Jones, D.N.C., 2010. Further neurochemical and behavioural investigation of Brattleboro rats as a putative model of schizophrenia. J. Psychopharmacol. 24, 407–19.

Corda, M.G., Piras, G., Lecca, D., Fernández-Teruel, A., Driscoll, P., Giorgi, O., 2005. The psychogenetically selected Roman rat lines differ in the susceptibility to develop amphetamine sensitization. Behav. Brain Res. 157, 147–56.

Csomor, P.A., Stadler, R.R., Feldon, J., Yee, B.K., Geyer, M.A., Vollenweider, F.X., 2008. Haloperidol differentially modulates prepulse inhibition and P50 suppression in healthy humans stratified for low and high gating levels. Neuropsychopharmacology 33, 497–512.

Csomor, P.A., Yee, B.K., Feldon, J., Theodoridou, A., Studerus, E., Vollenweider, F.X., 2009. Impaired prepulse inhibition and prepulse-elicited reactivity but intact reflex circuit excitability in unmedicated schizophrenia patients: a comparison with healthy subjects and medicated schizophrenia patients. Schizophr. Bull. 35, 244–55.

D'Angio, M., Serrano, A., Driscoll, P., Scatton, B., 1988. Stressful environmental stimuli increase extracellular DOPAC levels in the prefrontal cortex of hypoemotional (Roman high-avoidance) but not hyperemotional (Roman low-avoidance) rats. An in vivo voltammetric study. Brain Res. 451, 237–247.

Davis, M., 1984. The Mammalian Startle Response. In: Neural Mechanisms of Startle Behavior. Springer US, Boston, pp. 287–351.

Del Arco, A., Mora, F., Mohammed, A.H., Fuxe, K., 2007. Stimulation of D2 receptors in the prefrontal cortex reduces PCP-induced hyperactivity, acetylcholine release and dopamine metabolism in the nucleus accumbens. J. Neural Transm. 114, 185–193.

Del Arco, A., Mora, F., 2008. Prefrontal cortex-nucleus accumbens interaction: In vivo modulation by dopamine and glutamate in the prefrontal cortex. Pharmacol. Biochem. Behav, 90, 226-35

Del Arco, A., Mora, F., 2009. Neurotransmitters and prefrontal cortex-limbic system interactions: Implications for plasticity and psychiatric disorders. Journal of Neural Transmission, 116, 941–952.

Del Río, C., Oliveras, I., Cañete, T., Blázquez, G., Tobeña, A., Fernández-Teruel, A., 2014. Genetic rat models of schizophrenia-relevant symptoms. World J. Neurosci. 4, 261–278.

Del-Bel, E.A., Joca, S.R.L., Padovan, C.M., Guimarães, F.S., 2002. Effects of isolation-rearing on serotonin-1A and M1-muscarinic receptor messenger RNA expression in the hipocampal formation of rats, Neuroscience Letters, 332, 123–6.

Díaz-Morán, S., Palència, M., Mont-Cardona, C., Cañete, T., Blázquez, G., Martínez-Membrives, E., López-Aumatell, R., Tobeña, A., Fernández-Teruel, A., 2012. Coping style and stress hormone responses in genetically heterogeneous rats: comparison with the Roman rat strains. Behav. Brain Res. 228, 203–10.

Díaz-Morán, S., Martínez-Membrives, E., López-Aumatell, R., Cañete, T., Blázquez, G., Palencia, M., Mont-Cardona, C., Estanislau, C., Tobeña, A., Fernández-Teruel, A., 2013a. What can we learn on rodent fearfulness/anxiety from the genetically heterogeneous NIH-HS rat stock? Open J. Psychiatry, 3, 238–250.

Díaz-Morán, S., Palència, M., Mont-Cardona, C., Cañete, T., Blázquez, G., Martínez-Membrives, E., López-Aumatell, R., Sabariego, M., Donaire, R., Morón, I., Torres, C., Martínez-Conejero, J.A., Tobeña, A., Esteban, F.J., Fernández-Teruel, A., 2013b. Gene expression in amygdala as a function of differential trait anxiety levels in genetically heterogeneous NIH-HS rats. Behav. Brain Res. 252, 422–431.

Díaz-Morán, S., Palència, M., Mont-Cardona, C., Cañete, T., Blázquez, G., Martínez-Membrives, E., López-Aumatell, R., Sabariego, M., Donaire, R., Morón, I., Torres, C., Martínez-Conejero, J.A., Tobeña, A., Esteban, F.J., Fernández-Teruel, A., 2013c. Gene expression in hippocampus as a function of differential trait anxiety levels in genetically heterogeneous NIH-HS rats. Behav. Brain Res. 257, 129–139.

Domeney, A., Feldon, J., 1998. The Disruption of Prepulse Inhibition by Social Isolation in the Wistar Rat: How Robust Is the Effect? Pharmacol. Biochem. Behav. 59, 883–890.

Driscoll P, B.K., 1982. Behavioural, emotional and neurochemical profiles of rats selected for extreme differences in active, two-way avoidance performance, In: Genetics of the Brain. Elsevier, Amsterdam, pp. 95–123.

Driscoll, P., Ferré, P., Fernández-Teruel, A., Tobeña, A., Escorihuela, R.M., Levi de Stein, M., Wolfman, C., Medina, J., 1995. Effects of prenatal diazepam on two-way avoidance behavior, swimming navigation and brain levels of benzodiazepine-like molecules in male roman high- and low-avoidance rats. Psychopharmacology (Berl). 122, 51–57.

Driscoll, P., Fernàndez-Teruel, A., Corda, M.G., Giorgi, O., Steimer, T., 2009. Some guidelines for defining personality differences in rats. In: Handbook of Behavior Genetics. Springer, New York, pp. 281–300.

Eilam, D., Szechtman, H., 1989. Biphasic effect of D-2 agonist quinpirole on locomotion and movements. Eur. J. Pharmacol. 161, 151–7.

Elert, E., 2014. Aetiology: Searching for schizophrenia's roots. Nature 508, S2–S3.

Ellenbroek, B.A., Van Den Kroonenberg, P.T., Cools, A.R., 1998. The effects of an early stressful life event on sensorimotor gating in adult rats. Schizophr. Res. 30, 251–260.

Ellenbroek, B.A., Karl, T., 2016. Chapter 18 – Genetic rat models for schizophrenia. In: Handbook of Behavioral Neuroscience. Elsevier, Amsterdam, pp. 303–324.

Escorihuela, R.M., Fernández-Teruel, A., 1998. Modelos animales en psicopatología y psicofarmacología: del análisis experimental de la conducta a la neurogenética. Psicol. Conductual 6, 165–191.

Escorihuela, R.M., Fernández-Teruel, A., Gil, L., Aguilar, R., Tobeña, A., Driscoll, P., 1999. Inbred Roman High- and Low-Avoidance Rats: Differences in anxiety, novelty-seeking, and shuttlebox behaviors. Physiol. Behav. 67, 19–26.

Esnal, A., Sánchez-González, A., Río-Álamos, C., Oliveras, I., Cañete, T., Blázquez, G., Tobeña, A., Fernández-Teruel, A., 2016. Prepulse inhibition and latent inhibition deficits in Roman high-avoidance vs. Roman low-avoidance rats: Modeling schizophrenia-related features. Physiol. Behav. 163, 267–273.

Fatemi, S.H., Pearce, D.A., Brooks, A.I., Sidwell, R.W., 2005. Prenatal viral infection in mouse causes differential expression of genes in brains of mouse progeny: A potential animal model for schizophrenia and autism. Synapse 57, 91–99.

Fattore, L., Piras, G., Corda, M.G., Giorgi, O., 2009. The Roman high- and low-avoidance rat lines differ in the acquisition, maintenance, extinction, and reinstatement of intravenous cocaine self-administration. Neuropsychopharmacology 34, 1091–101.

Fendt, M., Koch, M., 2013. Translational value of startle modulations. Cell Tissue Res. 354, 287–95.

Fernández-Teruel, A., Escorihuela, R.M., Castellano, B., González, B., Tobeña, A., 1997. Neonatal handling and environmental enrichment effects on emotionality, novelty/reward seeking, and age-related cognitive and hippocampal impairments: Focus on the roman rat lines. Behav. Genet. 27, 513–526.

Fernández-Teruel, A., Blázquez, G., Pérez, M., Aguilar, R., Cañete, T., Guitart, M., Giménez-Llort, L., Tobeña, A., 2006. Latent inhibition threshold in Roman high-avoidance rats: A psychogenetic model of abnormalities in attentional filter? Actas Españolas Psiquiatr. 34, 257–263.

Fijał, K., Popik, P., Nikiforuk, A., 2014. Co-administration of 5-HT6 receptor antagonists with clozapine, risperidone, and a 5-HT2A receptor antagonist: effects on prepulse inhibition in rats. Psychopharmacology (Berl). 231, 269–81.

Fomsgaard, L., Moreno, J.L., de la Fuente Revenga, M., Brudek, T., Adamsen, D., Río-Álamos, C., Saunders, J., Klein, A.B., Oliveras, I., Cañete, T., Blázquez, G., Tobeña, A., Fernández-Teruel, A., González-Maeso, J., Aznar, S., 2017. Differences in 5-HT2A and mGlu2 receptor expression levels and repressive epigenetic modifications at the 5-HT2A promoter region in the Roman Low- (RLA-I) and High- (RHA-I) Avoidance rat strains. Mol. Neurobiol. doi:10.1007/s12035-017-0457-y

Fone, K.C.F., Porkess, M.V., 2008. Behavioural and neurochemical effects of post-weaning social isolation in rodents—Relevance to developmental neuropsychiatric disorders. Neurosci. Biobehav. Rev. 32, 1087–1102.

Fribourg, M., Moreno, J.L., Holloway, T., Provasi, D., Baki, L., Mahajan, R., Park, G., Adney, S.K., Hatcher, C., Eltit, J.M., Ruta, J.D., Albizu, L., Li, Z., Umali, A., Shim, J., Fabiato, A., MacKerell, A.D., Brezina, V., Sealfon, S.C., Filizola, M., González-Maeso, J., Logothetis, D.E., 2011. Decoding the signaling of a GPCR heteromeric complex reveals a unifying mechanism of action of antipsychotic drugs. Cell 147, 1011–23.

Frith, C.D., Blakemore, S.-J., Wolpert, D.M., 2000. Explaining the symptoms of schizophrenia: Abnormalities in the awareness of action. Brain Res. Rev. 31, 357–363.

Fuster, J.M., 2009. Cortex and Memory: Emergence of a New Paradigm. J. Cogn. Neurosci. 21, 2047–2072.

Gaebel, W., Barch, D.M., Bustillo, J., Gur, R.E., Heckers, S., Malaspina, D., Owen, M.J., Schultz, S., Tsuang, M., Van Os, J., Carpenter, W., 2013. Definition and description of schizophrenia in the DSM-5. Schizophr. Res. 150, 3–10.

Garcia-Falgueras, A., Castillo-Ruiz, M.M., Put, T., Tobeña, A., Fernández-Teruel, A., 2012. Differential hippocampal neuron density between inbred Roman high- (low anxious) and low-avoidance (high anxious) rats. Neurosci. Lett. 522, 41–6.

Garelick, M.G., Storm, D.R., 2005. The relationship between memory retrieval and memory extinction. Proc. Natl. Acad. Sci. 102, 9091–2.

Geyer, M., Moghaddam, B., 2002. Animal models relevant to schizophrenia disorders. In Neuropsychopharmacology: The Fifth Generation of Progress. Lippincott, Williams, & Wilkins, Philadelphia, pp. 689–701.

Geyer, M.A., Krebs-Thomson, K., Braff, D.L., Swerdlow, N.R., 2001. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology (Berl). 156, 117–154.

Geyer, M.A., Ellenbroek, B., 2003. Animal behavior models of the mechanisms underlying antipsychotic atypicality. Prog. Neuro-Psychopharmacology Biol. Psychiatry 27, 1071–1079.

Geyer, M.A., 2008. Developing translational animal models for symptoms of schizophrenia or bipolar mania. Neurotox. Res. 14, 71–78.

Geyer, M.A., Vollenweider, F.X., 2008. Serotonin research: contributions to understanding psychoses. Trends Pharmacol. Sci. 29, 445–53.

Geyer, M.A., Olivier, B., Joëls, M., Kahn, R.S., 2012. From antipsychotic to anti-schizophrenia drugs: role of animal models. Trends Pharmacol. Sci. 33, 515–21.

Giakoumaki, S.G., Bitsios, P., Frangou, S., 2006. The level of prepulse inhibition in healthy individuals may index cortical modulation of early information processing. Brain Res. 1078, 168–170.

Giakoumaki, S.G., Roussos, P., Bitsios, P., 2008. Improvement of prepulse inhibition and executive function by the COMT inhibitor tolcapone depends on COMT Val158Met polymorphism. Neuropsychopharmacology 33, 3058–3068.

Giménez-Llort, L., Cañete, T., Guitart-Masip, M., Fernández-Teruel, A., Tobeña, A., 2005. Two distinctive apomorphine-induced phenotypes in the Roman high- and low-avoidance rats. Physiol. Behav. 86, 458–66.

Giorgi, O., Lecca, D., Piras, G., Driscoll, P., Corda, M.G., 2003. Dissociation between mesocortical dopamine release and fear-related behaviours in two psychogenetically selected lines of rats that differ in coping strategies to aversive conditions. Eur. J. Neurosci. 17, 2716–2726.

Giorgi, O., Piras, G., Corda, M.G., 2007. The psychogenetically selected Roman high- and low-avoidance rat lines: A model to study the individual vulnerability to drug addiction. Neurosci. Biobehav. Rev. 31, 148–163.

Goghari, V.M., Sponheim, S.R., MacDonald, A.W., III, 2010. The functional neuroanatomy of symptom dimensions in schizophrenia: a qualitative and quantitative review of a persistent question. Neurosci. Biobehav. Rev. 34, 468–86.

Goldberg, E., 2009. The new executive brain: frontal lobes in a complex world. Oxford University Press, Oxford.

Goldman-Rakic, P.S., Selemon, L.D., Schwartz, M.L., 1984. Dual pathways connecting the dorsolateral prefrontal cortex with the hippocampal formation and parahippocampal cortex in the rhesus monkey. Neuroscience 12, 719–43.

González-Maeso, J., Weisstaub, N. V., Zhou, M., Chan, P., Ivic, L., Ang, R., Lira, A., Bradley-Moore, M., Ge, Y., Zhou, Q., Sealfon, S.C., Gingrich, J.A., 2007. Hallucinogens recruit specific cortical 5-HT2A receptor-mediated signaling pathways to affect behavior. Neuron 53, 439–452.

González-Maeso, J., Ang, R.L., Yuen, T., Chan, P., Weisstaub, N. V., López-Giménez, J.F., Zhou, M., Okawa, Y., Callado, L.F., Milligan, G., Gingrich, J.A., Filizola, M., Meana, J.J., Sealfon, S.C., 2008. Identification of a serotonin/glutamate receptor complex implicated in psychosis. Nature 452, 93–97.

González-Maeso, J., Sealfon, S.C., 2009. Psychedelics and schizophrenia. Trends Neurosci. 32, 225–32.

Gøtzsche, P.C., 2014. Medicamentos que matan y crimen organizado: cómo las grandes farmacéuticas han corrompido el sistema de salud. Los Libros del Lince, Barcelona.

Gøtzsche, P.C., 2015. Deadly psychiatry and organised denial. ArtPeople, Copenhague.

Greenwood, T.A., Light, G.A., Swerdlow, N.R., Calkins, M.E., Green, M.F., Gur, R.E., Gur, R.C., Lazzeroni, L.C., Nuechterlein, K.H., Olincy, A., Radant, A.D., Seidman, L.J., Siever, L.J., Silverman, J.M., Stone, W.S., Sugar, C.A., Tsuang, D.W., Tsuang, M.T., Turetsky, B.I., Freedman, R., Braff, D.L., 2016. Gating deficit heritability and correlation with increased clinical severity in schizophrenia patients with positive family history. Am. J. Psychiatry 173, 385–391.

Guitart-Masip, M., Johansson, B., Fernández-Teruel, A., Cañete, T., Tobeña, A., Terenius, L., Giménez-Llort, L., 2006. Divergent anatomical pattern of D1 and D3 binding and dopamine- and cyclic AMP-regulated phosphoprotein of 32 kDa mRNA expression in the Roman rat strains: Implications for drug addiction. Neuroscience 142, 1231–43.

Guitart-Masip, M., Johansson, B., Cañete, T., Fernández-Teruel, A., Tobeña, A., Terenius, L., Giménez-Llort, L., 2008a. Regional adaptations in PSD-95, NGFI-A and secretogranin gene transcripts related to vulnerability to behavioral sensitization to amphetamine in the Roman rat strains. Neuroscience 151, 195–208.

Guitart-Masip, M., Johansson, B., Fernández-Teruel, A., Tobeña, A., Giménez-Llort, L., 2008b. Divergent effect of the selective D3 receptor agonist pd-128,907 on locomotor activity in Roman high- and low-avoidance rats: relationship to NGFI-A gene expression in the Calleja islands. Psychopharmacology (Berl). 196, 39–49.

Hadamitzky, M., Harich, S., Koch, M., Schwabe, K., 2007. Deficient prepulse inhibition induced by selective breeding of rats can be restored by the dopamine D2 antagonist haloperidol. Behav. Brain Res. 177, 364–7.

Hagan, J.J., Jones, D.N.C., 2005. Predicting Drug Efficacy for Cognitive Deficits in Schizophrenia. Schizophr. Bull. 31, 830–853.

Halberstadt, A.L., van der Heijden, I., Ruderman, M.A., Risbrough, V.B., Gingrich, J.A., Geyer, M.A., Powell, S.B., 2009. 5-HT(2A) and 5-HT(2C) receptors exert opposing effects on locomotor activity in mice. Neuropsychopharmacology 34, 1958–67.

Halberstadt, A.L., Buell, M.R., Price, D.L., Geyer, M.A., 2012. Differences in the locomotor-activating effects of indirect serotonin agonists in habituated and non-habituated rats. Pharmacol. Biochem. Behav. 102, 88–94.

Halberstadt, A.L., Geyer, M.A., 2013. Serotonergic hallucinogens as translational models relevant to schizophrenia. Int. J. Neuropsychopharmacol. 16, 2165–80.

Hansen, C., Spuhler, K., 1984. Development of the National Institutes of Health Genetically Heterogeneous Rat Stock. Alcohol. Clin. Exp. Res. 8, 477–479.

Harrison, P.J., Owen, M.J., 2003. Genes for schizophrenia? Recent findings and their pathophysiological implications. Lancet 361, 417–419.

Harrison, P.J., Weinberger, D.R., 2005. Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. Mol. Psychiatry 10, 40–68.

Harvey, P.D., Green, M.F., McGurk, S.R., Meltzer, H.Y., 2003. Changes in cognitive functioning with risperidone and olanzapine treatment: a large-scale, double-blind, randomized study. Psychopharmacology (Berl). 169, 404–411.

Heckers, S., Konradi, C., 2010. Hippocampal pathology in schizophrenia. Curr. Top. Behav. Neurosci. 4, 529–53.

Hill, K., Mann, L., Laws, K.R., Stephenson, C.M.E., Nimmo-Smith, I., McKenna, P.J., 2004. Hypofrontality in schizophrenia: a meta-analysis of functional imaging studies. Acta Psychiatr. Scand. 110, 243–256.

Homberg, J.R., Olivier, J.D.A., Smits, B.M.G., Mul, J.D., Mudde, J., Verheul, M., Nieuwenhuizen, O.F.M., Cools, A.R., Ronken, E., Cremers, T., Schoffelmeer, A.N.M., Ellenbroek, B.A., Cuppen, E., 2007. Characterization of the serotonin transporter knockout rat: A selective change in the functioning of the serotonergic system. Neuroscience 146, 1662–1676.

Honea, R., Crow, T.J., Passingham, D., Mackay, C.E., 2005. Regional deficits in brain volume in schizophrenia: A meta-analysis of voxel-based morphometry studies. Am. J. Psychiatry 162, 2233–2245.

Howes, O.D., Kambeitz, J., Kim, E., Stahl, D., Slifstein, M., Abi-Dargham, A., Kapur, S., 2012. The nature of dopamine dysfunction in schizophrenia and what this means for treatment. Arch. Gen. Psychiatry 69, 776–86.

Ion, R.M., Beer, M.D., 2002. The British reaction to dementia praecox 1893-1913. Part 2. Hist. Psychiatry 13, 419–31.

Jentsch, J.D., Taylor, J.R., Roth, R.H., 1998. Subchronic phencyclidine administration increases mesolimbic dopaminergic system responsivity and augments stress- and psychostimulant- induced hyperlocomotion. Neuropsychopharmacology 19, 105–113.

Jentsch, J., Roth, R.H., 1999. The neuropsychopharmacology of phencyclidine from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 20, 201–225.

Johannesson, M., Lopez-Aumatell, R., Stridh, P., Diez, M., Tuncel, J., Blázquez, G., Martinez-Membrives, E., Cañete, T., Vicens-Costa, E., Graham, D., Copley, R.R., Hernandez-Pliego, P., Beyeen, A.D., Ockinger, J., Fernández-Santamaría, C., Gulko, P.S., Brenner, M., Tobeña, A., Guitart-Masip, M., Giménez-Llort, L., Dominiczak, A., Holmdahl, R., Gauguier, D., Olsson, T., Mott, R., Valdar, W., Redei, E.E., Fernández-Teruel, A., Flint, J., 2009. A resource for the simultaneous high-resolution mapping of multiple quantitative trait loci in rats: the NIH heterogeneous stock. Genome Res. 19, 150–8.

Jones, C.A., Watson, D.J.G., Fone, K.C.F., 2011. Animal models of schizophrenia. Br. J. Pharmacol. 164, 1162–94.

Kantrowitz, J.T., Javitt, D.C., 2010. N-methyl-d-aspartate (NMDA) receptor dysfunction or dysregulation: the final common pathway on the road to schizophrenia? Brain Res. Bull. 83, 108–21.

Kegeles, L.S., Martinez, D., Kochan, L.D., Hwang, D.-R., Huang, Y., Mawlawi, O., Suckow, R.F., Van Heertum, R.L., Laruelle, M., 2002. NMDA antagonist effects on striatal dopamine release: Positron emission tomography studies in humans. Synapse 43, 19–29.

Kegeles, L.S., Abi-Dargham, A., Frankle, W.G., Gil, R., Cooper, T.B., Slifstein, M., Hwang, D.-R., Huang, Y., Haber, S.N., Laruelle, M., 2010. Increased synaptic dopamine function in associative regions of the striatum in schizophrenia. Arch. Gen. Psychiatry 67, 231–9.

Kesner, R.P., Hunt, M.E., Williams, J.M., Long, J.M., 1996. Prefrontal cortex and working memory for spatial response, spatial location, and visual object information in the rat. Cereb. Cortex 6, 311–8.

Kinon, B.J., Zhang, L., Millen, B.A., Osuntokun, O.O., Williams, J.E., Kollack-Walker, S., Jackson, K., Kryzhanovskaya, L., Jarkova, N., HBBI Study Group, 2011. A multicenter, inpatient, phase 2, double-blind, placebo-controlled dose-ranging study of LY2140023 monohydrate in patients with DSM-IV schizophrenia. J. Clin. Psychopharmacol. 31, 349–55.

Kishi, T., Tsunoka, T., Ikeda, M., Kitajima, T., Kawashima, K., Okochi, T., Okumura, T., Yamanouchi, Y., Kinoshita, Y., Ujike, H., Inada, T., Yamada, M., Uchimura, N., Sora, I., Iyo, M., Ozaki, N., Iwata, N., 2010. Serotonin 1A receptor gene is associated with Japanese methamphetamine-induced psychosis patients. Neuropharmacology 58, 452–456.

Klein, A.B., Ultved, L., Adamsen, D., Santini, M.A., Tobeña, A., Fernández-Teruel, A., Flores, P., Moreno, M., Cardona, D., Knudsen, G.M., Aznar, S., Mikkelsen, J.D., 2014. 5-HT2A and mGlu2 receptor binding levels are related to differences in impulsive behavior in the Roman Low- (RLA) and High- (RHA) avoidance rat strains. Neuroscience 263, 36–45.

Knapp, D.J., Daws, L.C., Overstreet, D.H., 2014. Behavioral characteristics of pharmacologically selected lines of rats: Relevance to Depression. World J. Neurosci. 04, 225–239.

Koch, M., 2013. Chapter 7 – Clinical relevance of animal models of schizophrenia. In: Supplements to Clinical Neurophysiology. Elsevier, Amsterdam, pp. 113–120.

Koenig, J.I., Kirkpatrick, B., Lee, P., 2002. Glucocorticoid hormones and early brain development in schizophrenia. Neuropsychopharmacology 27, 309–318.

Kohl, S., Heekeren, K., Klosterkötter, J., Kuhn, J., 2013. Prepulse inhibition in psychiatric disorders – Apart from schizophrenia. J. Psychiatr. Res. 47, 445–452.

Kraepelin, E., 1893. *Psychiatrie,* Abel, Leipzig.

Kristiansen, L. V., Huerta, I., Beneyto, M., Meador-Woodruff, J.H., 2007. NMDA receptors and schizophrenia. Curr. Opin. Pharmacol. 7, 48–55

Lambe, E.K., Fillman, S.G., Webster, M.J., Shannon Weickert, C., 2011. Serotonin receptor expression in human prefrontal cortex: Balancing excitation and inhibition across postnatal development. PLoS One 6, e22799.

Lapiz, M.D.S., Fulford, A., Muchimapura, S., Mason, R., Parker, T., Marsden, C.A., 2003. Influence of postweaning social isolation in the rat on brain development, conditioned behavior, and neurotransmission. Neurosci. Behav. Physiol. 33, 13–29.

Laruelle, M., Abi-Dargham, A., van Dyck, C.H., Gil, R., D'Souza, C.D., Erdos, J., McCance, E., Rosenblatt, W., Fingado, C., Zoghbi, S.S., Baldwin, R.M., Seibyl, J.P., Krystal, J.H., Charney, D.S., Innis, R.B., 1996. Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. Proc. Natl. Acad. Sci. 93, 9235–9240.

Laruelle, M., Kegeles, L.S., Abi-Dargham, A., 2003. Glutamate, dopamine, and schizophrenia from pathophysiology to treatment. Ann. N.Y. Acad. Sci. 1003, 138–158.

Leucht, S., Cipriani, A., Spineli, L., Mavridis, D., Örey, D., Richter, F., Samara, M., Barbui, C., Engel, R.R., Geddes, J.R., Kissling, W., Stapf, M.P., Lässig, B., Salanti, G., Davis, J.M., 2013. Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: a multiple-treatments meta-analysis. Lancet 382, 951–962.

Levin, E.D., Petro, A., Caldwell, D.P., 2005. Nicotine and clozapine actions on pre-pulse inhibition deficits caused by N-methyl-D-aspartate (NMDA) glutamatergic receptor blockade. Prog. Neuropsychopharmacol. Biol. Psychiatry 29, 581–6.

Lieberman, J.A., Stroup, T.S., Mcevoy, J.P., Swartz, M.S., Rosenheck, R.A., Perkins, D.O., Keefe, R.S.E., Davis, S.M., Davis, C.E., Lebowitz, B.D., Severe, J., Hsiao, J.K., 2005. Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. N Engl. J Med. 353, 1209–23.

Liu, Y.P., Lin, Y.L., Chuang, C.H., Kao, Y.C., Chang, S.T., Tung, C.S., 2009 Alpha adrenergic modulation on effects of norepinephrine transporter inhibitor reboxetine in five-choice serial reaction time task. J Biomed Sci. 16:72.

Liu, W., Downing, A.C.M., Munsie, L.M., Chen, P., Reed, M.R., Ruble, C.L., Landschulz, K.T., Kinon, B.J., Nisenbaum, L.K., 2012. Pharmacogenetic analysis of the mGlu2/3 agonist LY2140023 monohydrate in the treatment of schizophrenia. Pharmacogenomics J. 12, 246–254.

Lodge, D.J., Grace, A.A., 2008. Hippocampal dysfunction and disruption of dopamine system regulation in an animal model of schizophrenia. Neurotox. Res. 14, 97–104.

López-Aumatell, R., Guitart-Masip, M., Vicens-Costa, E., Gimenez-Llort, L., Valdar, W., Johannesson, M., Flint, J., Tobeña, A., Fernández-Teruel, A., 2008. Fearfulness in a large N/Nih genetically heterogeneous rat stock: Differential profiles of timidity and defensive flight in males and females. Behav. Brain Res. 188, 41–55.

López Aumatell, R., Vicens Costa, E., Guitart Masip, M., Martínez Membrives, E., Valdar, W., Johannesson, M., Cañete, T., Blázquez, G., Giménez Llort, L., Flint, J., Tobeña, A., Fernández-Teruel, A., 2009. Ansiedad en ratas genéticamente heterogéneas: hacia la identificación de genes para caracteres conductuales cuantitativos. Ansiedad y estrés, 15, 67-84.

López-Aumatell, R., Blázquez, G., Gil, L., Aguilar, R., Cañete, T., Giménez-Llort, L., Tobeña, A., Fernández-Teruel, A., 2009a. The Roman High- and Low-Avoidance rat strains differ in fear-potentiated startle and classical aversive conditioning. Psicothema 21, 27–32.

López-Aumatell, R., Vicens-Costa, E., Guitart-Masip, M., Martínez-Membrives, E., Valdar, W., Johannesson, M., Cañete, T., Blázquez, G., Driscoll, P., Flint, J., Tobeña, A., Fernández-Teruel, A., 2009b. Unlearned anxiety predicts learned fear: A comparison among heterogeneous rats and the Roman rat strains. Behav. Brain Res. 202, 92–101.

López-Aumatell, R., Martínez-Membrives, E., Vicens-Costa, E., Cañete, T., Blázquez, G., Mont-Cardona, C., Johannesson, M., Flint, J., Tobeña, A., Fernández-Teruel, A., 2011. Effects of environmental and physiological covariates on sex differences in unconditioned and conditioned anxiety and fear in a large sample of genetically heterogeneous (N/Nih-HS) rats. Behav. Brain Funct. 7, 48.

Luby, E.D., Cohen, B.D., Rosenbaum, G., Gottlieb, J.S., Kelley, R., 1959. Study of a new schizophrenomimetic drug; sernyl. A. M. A. Arch. Neurol. psychiatry 81, 363–369.

Mansbach, R.S., Geyer, M.A., Braff, D.L., 1988. Dopaminergic stimulation disrupts sensorimotor gating in the rat. Psychopharmacology (Berl). 94, 507–14.

Mansbach, R.S., Braff, D.L., Geyer, M.A., 1989. Prepulse inhibition of the acoustic startle response is disrupted by N-ethyl-3,4-methylenedioxyamphetamine (MDEA) in the rat. Eur. J. Pharmacol. 167, 49–55.

Manzo, L., Gómez, M.J., Callejas-Aguilera, J.E., Fernández-Teruel, A., Papini, M.R., Torres, C., 2014. Anti-anxiety self-medication induced by incentive loss in rats. Physiol. Behav. 123, 86–92.

Marcotte, E.R., Pearson, D.M., Srivastava, L.K., 2001. Animal models of schizophrenia: a critical review. J. Psychiatry Neurosci. 26, 395–410.

Martínez-Membrives, E., López-Aumatell, R., Blázquez, G., Cañete, T., Tobeña, A., Fernández-Teruel, A., 2015. Spatial learning in the genetically heterogeneous NIH-HS rat stock and RLA-I/RHA-I rats: Revisiting the relationship with unconditioned and conditioned anxiety. Physiol. Behav. 144, 15–25.

McCarley, R.W., Wible, C.G., Frumin, M., Hirayasu, Y., Levitt, J.J., Fischer, I.A., Shenton, M.E., 1999. MRI anatomy of schizophrenia. Biol. Psychiatry 45, 1099–119.

McGhie, A., Chapman, J., 1961. Disorders of attention and perception in early schizophrenia. Br. J. Med. Psychol. 34, 103–116.

McLean, S., Grayson, B., Harris, M., Protheroe, C., Woolley, M., Neill, J., 2010. Isolation rearing impairs novel object recognition and attentional set shifting performance in female rats. J. Psychopharmacol. 24, 57–63.

Meltzer, H.Y., 1997. Treatment-resistant schizophrenia - The role of clozapine. Curr. Med. Res. Opin. 14, 1–20.

Meltzer, H.Y., Horiguchi, M., Massey, B.W., 2011. The role of serotonin in the NMDA receptor antagonist models of psychosis and cognitive impairment. Psychopharmacology (Berl). 213, 289–305.

Meneses, A., Perez-Garcia, G., Ponce-Lopez, T., Tellez, R., Gallegos-Cari, A., Castillo, C., 2011. Spontaneously hypertensive rat (SHR) as an animal model for ADHD: a short overview. Rev. Neurosci. 22, 365–371.

Meyer, U., Feldon, J., 2009. Neural basis of psychosis-related behaviour in the infection model of schizophrenia. Behav. Brain Res. 204, 322–334.

Meyer, U., Feldon, J., 2010. Epidemiology-driven neurodevelopmental animal models of schizophrenia. Prog. Neurobiol. 90, 285–326.

Meyer-Lindenberg, A., 2010. From maps to mechanisms through neuroimaging of schizophrenia. Nature 468, 194–202.

Meyza, K.Z., Boguszewski, P.M., Nikolaev, E., Zagrodzka, J., 2009. Diverse sensitivity of RHA/Verh and RLA/Verh rats to emotional and spatial aspects of a novel environment as a result of a distinct pattern of neuronal activation in the fear/anxiety circuit. Behav. Genet. 39, 48–61.

Millan, M.J., Andrieux, A., Bartzokis, G., Cadenhead, K., Dazzan, P., Fusar-Poli, P., Gallinat, J., Giedd, J., Grayson, D.R., Heinrichs, M., Kahn, R., Krebs, M.-O., Leboyer, M., Lewis, D., Marin, O., Marin, P., Meyer-Lindenberg, A., McGorry, P., McGuire, P., Owen, M.J., Patterson, P., Sawa, A., Spedding, M., Uhlhaas, P., Vaccarino, F., Wahlestedt, C., Weinberger, D., 2016. Altering the course of schizophrenia: progress and perspectives. Nat. Rev. Drug Discov. 15, 485–515.

Mirsky, A.F., Primac, D.W., Marsan, C.A., Rosvold, H.E., Stevens, J.R., 1960. A comparison of the psychological test performance of patients with focal and nonfocal epilepsy. Exp. Neurol. 2, 75–89.

Modinos, G., Allen, P., Grace, A.A., McGuire, P., 2015. Translating the MAM model of psychosis to humans. Trends Neurosci. 38, 129–38.

Moreno, J.L., Fribourg, M., González, Y.J., 2010a. Bases bioquímicas de la esquizofrenia Mente y Cerebro, 44,18–27.

Moreno, M., Cardona, D., Gómez, M.J., Sánchez-Santed, F., Tobeña, A., Fernández-Teruel, A., Campa, L., Suñol, C., Escarabajal, M.D., Torres, C., Flores, P., 2010b. Impulsivity characterization in the Roman high- and low-avoidance rat strains: behavioral and neurochemical differences. Neuropsychopharmacology 35, 1198–208.

Morris, R.G.M., Anderson, E., Lynch, G.S., Baudry, M., 1986a. Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. Nature 319, 774–776.

Morris, R.G.M., Hagan, J.J., Rawlins, J.N., 1986b. Allocentric spatial learning by hippocampectomised rats: a further test of the “spatial mapping” and “working memory” theories of hippocampal function. Q. J. Exp. Psychol. B. 38, 365–95.

Mosolov, S.N., Potapov, A. V, Ushakov, U. V, 2012. Remission in schizophrenia: results of cross-sectional with 6-month follow-up period and 1-year observational therapeutic studies in an outpatient population. Ann. Gen. Psychiatry 11, 1.

Muchimapura, S., Mason, R., Marsden, C.A., 2003. Effect of isolation rearing on pre- and post-synaptic serotonergic function in the rat dorsal hippocampus. Synapse 47, 209–17.

Muguruza, C., Moreno, J.L., Umali, A., Callado, L.F., Meana, J.J., González-Maeso, J., 2013. Dysregulated 5-HT2A receptor binding in postmortem frontal cortex of schizophrenic subjects. Eur. Neuropsychopharmacol. 23, 852–864.

Muguruza, C., Meana, J.J., Callado, L.F., 2016. Group II metabotropic glutamate receptors as targets for novel antipsychotic drugs. Front. Pharmacol. 7, 130.

Neill, J.C., Barnes, S., Cook, S., Grayson, B., Idris, N.F., McLean, S.L., Snigdha, S., Rajagopal, L., Harte, M.K., 2010. Animal models of cognitive dysfunction and negative symptoms of schizophrenia: focus on NMDA receptor antagonism. Pharmacol. Ther. 128, 419–32.

Nestler, E.J., Hyman, S.E., 2010. Animal models of neuropsychiatric disorders. Nat. Neurosci. 13, 1161–9.

Nil, R., Bättig, K., 1981. Spontaneous maze ambulation and Hebb-Williams learning in Roman High-Avoidance and Roman Low-Avoidance rats. Behav. Neural Biol. 33, 465–475.

Niznikiewicz, M.A., Kubicki, M., Shenton, M.E., 2003. Recent structural and functional imaging findings in schizophrenia. Curr. Opin. Psychiatry 16, 123–147.

Pandya, D.N., Yeterian, E.H., 1990. Prefrontal cortex in relation to other cortical areas in rhesus monkey: architecture and connections. Prog. Brain Res. 85, 63–94.

Patil, S.T., Zhang, L., Martenyi, F., Lowe, S.L., Jackson, K.A., Andreev, B. V, Avedisova, A.S., Bardenstein, L.M., Gurovich, I.Y., Morozova, M.A., Mosolov, S.N., Neznanov, N.G., Reznik, A.M., Smulevich, A.B., Tochilov, V.A., Johnson, B.G., Monn, J.A., Schoepp, D.D., 2007. Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized Phase 2 clinical trial. Nat. Med. 13, 1102–1107.

Piras, G., Giorgi, O., Corda, M.G., 2010. Effects of antidepressants on the performance in the forced swim test of two psychogenetically selected lines of rats that differ in coping strategies to aversive conditions. Psychopharmacology (Berl). 211, 403–414.

Powell, S.B., Geyer, M.A., 2002. Developmental markers of psychiatric disorders as identified by sensorimotor gating. Neurotox. Res. 4, 489–502.

Powell, S.B., Swerdlow, N.R., Pitcher, L.K., Geyer, M.A., 2002. Isolation rearing-induced deficits in prepulse inhibition and locomotor habituation are not potentiated by water deprivation. Physiol. Behav. 77, 55–64.

Preece, M.., Dalley, J.., Theobald, D.E.., Robbins, T.., Reynolds, G.., 2004. Region specific changes in forebrain 5-hydroxytryptamine1a and 5-hydroxytryptamine2a receptors in isolation-reared rats: an in vitro autoradiography study. Neuroscience 123, 725–732.

Rigdon, G.C., Weatherspoon, J.K., 1992. 5-Hydroxytryptamine 1a receptor agonists block prepulse inhibition of acoustic startle reflex. J. Pharmacol. Exp. Ther. 263, 486–493

Río-Ȧlamos, C., Oliveras, I., Cañete, T., Blázquez, G., Martínez-Membrives, E., Tobeña, A., Fernández-Teruel, A., 2015. Neonatal handling decreases unconditioned anxiety, conditioned fear, and improves two-way avoidance acquisition: a study with the inbred Roman high (RHA-I) - and low-avoidance (RLA-I) rats of both sexes. Front. Behav. Neurosci. 9,174.

Río-Álamos, C., Oliveras, I., Piludu, M.A., Gerbolés, C., Cañete, T., Blázquez, G., Lope-Piedrafita, S., Martínez-Membrives, E., Torrubia, R., Tobeña, A., Fernández-Teruel, A., 2017. Neonatal handling enduringly decreases anxiety and stress responses and reduces hippocampus and amygdala volume in a genetic model of differential anxiety: Behavioral-volumetric associations in the Roman rat strains. Eur. Neuropsychopharmacol. 27, 146–158.

Robbins, T.W., Everitt, B.J., Marston, H.M., Wilkinson, J., Jones, G.H., Page, K.J., 1989. Comparative effects of ibotenic acid- and quisqualic acid-induced lesions of the substantia innominata on attentional function in the rat: further implications for the role of the cholinergic neurons of the nucleus basalis in cognitive processes. Behav. Brain Res. 35, 221–40.

Ross, C.A., Margolis, R.L., Reading, S.A.J., Pletnikov, M., Coyle, J.T., 2006. Neurobiology of Schizophrenia. Neuron 52, 139–153.

Saha, K.B., Bo, L., Zhao, S., Xia, J., Sampson, S., Zaman, R.U., 2016. Chlorpromazine versus atypical antipsychotic drugs for schizophrenia. Cochrane Database Syst. Rev. 4, CD010631

Samara, M.T., Leucht, C., Leeflang, M.M., Anghelescu, I.-G., Chung, Y.-C., Crespo-Facorro, B., Elkis, H., Hatta, K., Giegling, I., Kane, J.M., Kayo, M., Lambert, M., Lin, C.-H., Möller, H.-J., Pelayo-Terán, J.M., Riedel, M., Rujescu, D., Schimmelmann, B.G., Serretti, A., Correll, C.U., Leucht, S., 2015. Early improvement as a predictor of later response to antipsychotics in schizophrenia: a diagnostic test review. Am. J. Psychiatry 172, 617–629.

Sams-Dodd, F., 1998. A test of the predictive validity of animal models of schizophrenia based on phencyclidine and d-amphetamine. Neuropsychopharmacology 18, 293–304.

Sanna, F., Corda, M.G., Melis, M.R., Piludu, M.A., Giorgi, O., Argiolas, A., 2014a. Male Roman high and low avoidance rats show different patterns of copulatory behaviour: comparison with Sprague Dawley rats. Physiol. Behav. 127, 27–36.

Sanna, F., Piludu, M.A., Corda, M.G., Argiolas, A., Giorgi, O., Melis, M.R., 2014b. Dopamine is involved in the different patterns of copulatory behaviour of Roman high and low avoidance rats: studies with apomorphine and haloperidol. Pharmacol. Biochem. Behav. 124, 211–9.

Sawa, A., Snyder, S.H., 2002. Schizophrenia: diverse approaches to a complex disease. Science 296, 692–5.

Sawa, A., 2009. Cortical development and glutamatergic dysregulation in schizophrenia. Biol. Psychiatry 66, 530–2.

Schneider K., 1959. Clinical Psychopathology, Grune & Stratton, New York.

Schwabe, K., Freudenberg, F., Koch, M., 2007. Selective breeding of reduced sensorimotor gating in Wistar rats. Behav. Genet. 37, 706–12.

Selten, M.M., Meyer, F., Ba, W., Vallès, A., Maas, D.A., Negwer, M., Eijsink, V.D., van Vugt, R.W.M., van Hulten, J.A., van Bakel, N.H.M., Roosen, J., van der Linden, R.J., Schubert, D., Verheij, M.M.M., Kasri, N.N., Martens, G.J.M., 2016. Increased GABAB receptor signaling in a rat model for schizophrenia. Sci. Rep. 6, 34240.

Shenton, M.E., Kikinis, R., Jolesz, F.A., Pollak, S.D., LeMay, M., Wible, C.G., Hokama, H., Martin, J., Metcalf, D., Coleman, M., McCarley, R.W., 1992. Abnormalities of the left temporal lobe and thought disorder in schizophrenia. N. Engl. J. Med. 327, 604–612.

Singer, P., Hauser, J., Lopez, L.Ll., Peleg-Raibstein, D., Feldon, J., Gargiulo, P.A., Yee, B.K., 2013. Prepulse inhibition predicts working memory performance whilst startle habituation predicts spatial reference memory retention in C57BL/6 mice. Behav. Brain Res. 242, 166–177.

Small, S.A., Schobel, S.A., Buxton, R.B., Witter, M.P., Barnes, C.A., 2011. A pathophysiological framework of hippocampal dysfunction in ageing and disease. Nat. Rev. Neurosci. 12, 585–601.

Stahl, S. M., 2000. Essential Psychopharmacology Neuroscientific Basis and Practical Applications. Cambridge University Press, Cambridge.

Steeds, H., Carhart-Harris, R.L., Stone, J.M., 2015. Drug models of schizophrenia. Ther. Adv. Psychopharmacol. 5, 43–58.

Steimer, T., Driscoll, P., Schulz, P.E., 1997. Brain metabolism of progesterone, coping behaviour and emotional reactivity in male rats from two psychogenetically selected lines. J. Neuroendocrinol. 9, 169–75.

Steimer, T., Driscoll, P., 2003. Divergent Stress Responses and Coping Styles in Psychogenetically Selected Roman High-(RHA) and Low-(RLA) Avoidance Rats: behavioural, neuroendocrine and developmental aspects. Stress 6, 87–100.

Steimer, T., Driscoll, P., 2005. Inter-individual vs line/strain differences in psychogenetically selected Roman High-(RHA) and Low-(RLA) Avoidance rats: neuroendocrine and behavioural aspects. Neurosci. Biobehav. Rev. 29, 99–112.

Swerdlow, N.R., Braff, D.L., Geyer, M.A., Koob, G.F., 1986. Central dopamine hyperactivity in rats mimics abnormal acoustic startle response in schizophrenics. Biol. Psychiatry 21, 23–33.

Swerdlow, N.R., Koob, G.F., Mowrer, O.H., 1987. Dopamine, schizophrenia, mania, and depression: Toward a unified hypothesis of cortico-striatopallido-thalamic function. Behav. Brain Sci. 10, 197–208.

Swerdlow, N.R., Caine, S.B., Geyer, M.A., 1992. Regionally selective effects of intracerebral dopamine infusion on sensorimotor gating of the startle reflex in rats. Psychopharmacology (Berl). 108, 189–195.

Swerdlow, N.R., Weber, M., Qu, Y., Light, G.A., Braff, D.L., 2008. Realistic expectations of prepulse inhibition in translational models for schizophrenia research. Psychopharmacology (Berl). 199, 331–388.

Tamminga, C., Holcomb, H., 2005. Phenotype of schizophrenia: a review and formulation. Mol. Psychiatry 10, 27–39.

Tandon, R., Gaebel, W., Barch, D.M., Bustillo, J., Gur, R.E., Heckers, S., Malaspina, D., Owen, M.J., Schultz, S., Tsuang, M., Van Os, J., Carpenter, W., 2013. Definition and description of schizophrenia in the DSM-5. Schizophr. Res. 150, 3–10.

Taylor, S.B., Taylor, A.R., Markham, J.A., Geurts, A.M., Kanaskie, B.Z., Koenig, J.I., 2011. Disruption of the neuregulin 1 gene in the rat alters HPA axis activity and behavioral responses to environmental stimuli. Physiol. Behav. 104, 205–14.

Tiihonen, J., Wahlbeck, K., 2006. Glutamatergic drugs for schizophrenia. Cochrane Database Syst. Rev. 2, CD003730.

Tobeña, A., Fernández-Teruel, A., 2010. Modelos animales en psiquitría. In: Tratado de psiquiatría. vol. I. Ars Médica, Barcelona. pp 414–426

Tournier, B.B., Steimer, T., Millet, P., Moulin-Sallanon, M., Vallet, P., Ibañez, V., Ginovart, N., 2013. Innately low D2 receptor availability is associated with high novelty-seeking and enhanced behavioural sensitization to amphetamine. Int. J. Neuropsychopharmacol. 16, 1819–1834.

Tseng, K.Y., Chambers, R.A., Lipska, B.K., 2009. The neonatal ventral hippocampal lesion as a heuristic neurodevelopmental model of schizophrenia. Behav. Brain Res. 204, 295–305.

Vaillancourt, C., Boksa, P., 2000. Birth insult alters dopamine-mediated behavior in a precocial species, the guinea pig. Implications for schizophrenia. Neuropsychopharmacology 23, 654–66.

van den Buuse, M., 2010. Modeling the positive symptoms of schizophrenia in genetically modified mice: pharmacology and methodology aspects. Schizophr. Bull. 36, 246–70.

van Haaren, F., 1993. Methods in behavioral pharmacology. Elsevier, Amsterdam.

van Os, J., Kapur, S., 2009. Schizophrenia. Lancet 374, 635–645.

Vargas, J.P., Díaz, E., Portavella, M., López, J.C., 2016. Animal models of maladaptive traits: disorders in sensorimotor gating and attentional quantifiable responses as possible endophenotypes. Front. Psychol. 7, 206.

Vollenweider, F.X., Geyer, M.A., 2001. A systems model of altered consciousness: integrating natural and drug-induced psychoses. Brain Res. Bull. 56, 495–507.

Wakuda, T., Matsuzaki, H., Suzuki, K., Iwata, Y., Shinmura, C., Suda, S., Iwata, K., Yamamoto, S., Sugihara, G., Tsuchiya, K.J., Ueki, T., Nakamura, K., Nakahara, D., Takei, N., Mori, N., 2008. Perinatal asphyxia reduces dentate granule cells and exacerbates methamphetamine-induced hyperlocomotion in adulthood. PLoS One 3, e3648.

Weiss, I.C., Feldon, J., 2001. Environmental animal models for sensorimotor gating deficiencies in schizophrenia: a review. Psychopharmacology (Berl). 156, 305–326.

Whishaw, I.Q., 1985. Formation of a place learning-set by the rat: A new paradigm for neurobehavioral studies. Physiol. Behav. 35, 139–143.

Wible, C.G., 2013. Hippocampal physiology, structure and function and the neuroscience of schizophrenia: a unified account of declarative memory deficits, working memory deficits and schizophrenic symptoms. Behav. Sci. 3, 298–315.

Wible, C.G., Anderson, J., Shenton, M.E., Kricun, A., Hirayasu, Y., Tanaka, S., Levitt, J.J., O'Donnell, B.F., Kikinis, R., Jolesz, F.A., McCarley, R.W., 2001. Prefrontal cortex, negative symptoms, and schizophrenia: an MRI study. Psychiatry Res. 108, 65–78.

Wible, C.G., Shenton, M.E., Hokama, H., Kikinis, R., Jolesz, F.A., Metcalf, D., McCarley, R.W., 1995. Prefrontal cortex and schizophrenia. Arch. Gen. Psychiatry 52, 279–288.

Willner, P., 1991. Behavioural models in psychopharmacology Cambridge University Press, Cambridge.

Winton-Brown, T.T., Fusar-Poli, P., Ungless, M.A., Howes, O.D., 2014. Dopaminergic basis of salience dysregulation in psychosis. Trends Neurosci. 37, 85–94.

Wischhof, L., Aho, H.E.A., Koch, M., 2012. DOI-induced deficits in prepulse inhibition in Wistar rats are reversed by mGlu2/3 receptor stimulation. Pharmacol. Biochem. Behav. 102, 6–12.

Wischhof, L., Koch, M., 2016. 5-HT2A and mGlu2/3 receptor interactions. Behav. Pharmacol. 27, 1–11.

Wood, C.M., Nicolas, C.S., Choi, S.-L., Roman, E., Nylander, I., Fernández-Teruel, A., Kiianmaa, K., Bienkowski, P., de Jong, T.R., Colombo, G., Chastagnier, D., Wafford, K.A., Collingridge, G.L., Wildt, S.J., Conway-Campbell, B.L., Robinson, E.S.J., Lodge, D., 2017. Prevalence and influence of cys407* Grm2 mutation in Hannover-derived Wistar rats: mGlu2 receptor loss links to alcohol intake, risk taking and emotional behaviour. Neuropharmacology. 115, 128–138

World Health Organization, 1992. International classification of diseases and related health problems, WHO, Geneva (10th revision).

Young, J.W., Powell, S.B., Risbrough, V., Marston, H.M., Geyer, M.A., 2009. Using the MATRICS to guide development of a preclinical cognitive test battery for research in schizophrenia. Pharmacol. Ther. 122, 150–202.

Zeier, H., Baettig, K., Driscoll, P., 1978. Acquisition of DRL-20 behavior in male and female, Roman high- and low-avoidance rats. Physiol. Behav. 20, 791–793.

19. Annex 1:

ESTUDI 4

Associations between prefrontal cortex serotonin 1A and 2A receptor expression and sensorimotor gating in Roman and genetically heterogeneous NIH-HS rats

(Manuscrit no publicat)

Associations between prefrontal cortex serotonin 1A and 2A receptor expression and sensorimotor gating in Roman and genetically heterogeneous NIH-HS rats

Ignasi Oliveras[1], Cristóbal Río-Alamos[1], Toni Cañete[1], Gloria Blázquez[1], Tomasz Brudek[2], Jonas Folke[2], Luna Fomsgaard[2], Javier González-Maeso[3], Adolf Tobeña[1], Susana Aznar[2], Alberto Fernández-Teruel[1]

[1] Departamento de Psiquiatria y Medicina Legal, Instituto de Neurociencias, Universidad Autonoma de Barcelona, Barcelona, Spain.

[2] Research Laboratory for Stereology and Neuroscience, Bispebjerg and Frederiksberg Hospitals, Copenhagen, Denmark.

[3] Department of Physiology and Biophysics, Virginia Commonwealth University School of Medicine, Richmond, VA 23298, USA.

ABSTRACT

The serotonin 1A and 2A receptors (5-HT1AR and 5-HT2AR) are involved in many cognitive and attentional processes. We have evaluated the gene expression of both receptors in the prefrontal cortex, hippocampus and striatum of the Roman rats, that exhibit different protein levels of those receptors and differential scores of prepulse inhibition of the startle response (PPI), and also of the genetically heterogeneous NIH-HS rats selected for their extreme (low vs. high) scores in PPI. Additionally, we have also carried out correlational and discriminant analyses to explore the association between gene expression and PPI levels in these rat strains. The results show that, compared to RLA-I rats, the RHA-Is have increased *HTR1A* and *HTR2A* expression in the prefrontal cortex and increased *HTR2A* in the striatum, whereas NIH-HS rats selected for their low levels of PPI show increased expression of the HTR1A gene in the prefrontal cortex. In the associational study we found that gene expression of both receptors is negatively associated with PPI and that *HTR1A* have a greater discriminant power.

Key words: Prepulse inhibition, serotonin 1A receptors, serotonin 2A receptors, Roman rat strains, genetically heterogeneous rats, schizophrenia

1. Introduction

Schizophrenia is a neuropsychiatric chronic disorder that affects 1% of the general population. It is defined by the psychotic symptoms (delusions and hallucinations), negative symptoms (avolition, anhedonia among others) and cognitive symptoms (mainly, working memory deficits and attention disturbances). Regarding the etiology of the disorder many authors have discussed the role that changes in the serotonin system could have in some phenotypes regarding schizophrenia. These features are usually related to the negative symptoms such as affective disturbances and emotional processing deficits (Selvaraj et al., 2014).

Prepulse Inhibition (PPI), defined as the reduction of the startle response elicited by a weaker stimulus that precedes a startling-stimulus, has been studied as an endophenotype related to schizophrenia and other disorders characterized by a disrupted sensorimotor gating (Kohl et al., 2013). In search of neurotransmitter systems involved on PPI some gene expression studies have shown that expression of comt (cathecolamines), grid2 (ionotropic glutamate receptor) and slc1a2 (astroglial glutamate transporter) in the medial prefrontal cortex (PFC) were positively correlated with PPI, while grid2 and slc1a2 expression in the nucleus accumbens were negatively correlated with PPI (Swerdlow et al., 2013a,b; Shilling et al., 2008). No expression differences of serotonin receptors (or serotonin-related genes) nor associations with PPI were evaluated in these studies.

The interest in serotonin 1A receptor (5-HT1AR) related to schizophrenia therapies has been growing in the recent years due to evidence that agonists of this receptor reverse depression and anxiety symptoms in animal models, suggesting that this receptor could also be implicated in the negative symptoms of schizophrenia. Additionally, several studies indicate that 5-HT1AR is enhanced in PFC from schizophrenic patients (see “Discussion”), it is implicated in neurogenesis and in decreasing the extrapyramidal effects induced by typical antipsychotics (McCreary and Newman-Tancredi, 2015). Furthermore, it has been seen that tandospirone (partial agonist at 5-HT1AR) normalizes memory deficits in schizophrenia. All of these evidences, and the development of new antipsychotics such as lurasidone and cariprazine (partial agonists at 5-HT1AR), could lead to the discovery of new and more efficient pharmacological therapies for this disorder (McCreary and Newman-Tancredi 2015).

The serotonin 2A receptor (5-HT2AR) has been implicated in the genesis, the treatment, the positive and negative symptoms present in schizophrenia, as well as in the reduction of extrapyramidal effects seen with atypical (relative to typical) antipsychotics (e.g. Geyer et al.

2001; Geyer and Vollenweider 2008; see "Discussion"). The administration of 5-HT2AR agonists such as mescaline, psilocybin or lysergic acid diethylamide (LSD) induce hallucinations through the activation of this receptor, and this action is reversed with some atypical antipsychotics. Atypical antipsychotics usually have antagonist activity for the D2 and the 5-HT2A receptors. Moreover, the DNA sequence variation of the encoding gene (*HTR2A*; 13q14-21) has also been linked to increased risk to develop schizophrenia, (Norton and Owen 2005) and also to the efficacy of the atypical antipsychotics (Arranz et al.,1995, 2000).

The Roman rat strains are bidirectionally selected and bred for their extreme performance in the two-way active avoidance task. Roman High-avoidance (RHA-I) rats acquire this task very rapidly while their Roman Low-avoidance (RLA-I) counterparts show extremely poor acquisition of the task (e.g. Driscoll et al., 1998, 2009; Escorihuela et al., 1999; Diaz-Moran et al., 2012; Rio-Alamos et al., 2015, 2016). Both strains show differences with respect to several co-selected phenotypes (other than two-way avoidance). With respect to schizophrenia-related features the Roman strains exhibit a clear difference in PPI (RHA < RLA) that has been reported and replicated many times in our laboratory (Del Rio et al., 2014, Esnal et al., 2016; Oliveras et al., 2015, 2016, 2017; Rio-Alamos et al., 2017) and, notably, RHA-I rats also show impaired latent inhibition and spatial working memory (Fernandez-Teruel et al., 2006; Esnal et al., 2016; Oliveras et al., 2015). Moreover, and perhaps related to these differential schizophrenia-relevant profiles, recent reports have shown that RHA-I rats present higher 5-HT1A and 5-HT2A binding levels in the PFC than their RLA-I counterparts, while the strains also differ in expression protein levels of the mGlu2/5-HT2A receptor complex (Klein et al., 2014; Wood et al., 2016; Fomsgaard et al., 2017), which has been involved in schizophrenia (Gonzalez-Maeso et al., 2008; see also "Discussion").

The genetically heterogeneous NIH-HS rat stock (i.e., "National Institutes of Health Genetically Heterogeneous Rat Stock") was developed by Hansen and Spuhler (1984) through an eight-way cross from eight inbred rat strains and they were bred for more than 60 generations. Due to their broad phenotypic variation and high degree of genetic recombination, they are used to study the genetic basis of many complex phenotypes, as they are considered to be a plausible parallel of a healthy human sample and to have higher translational power (e.g., Spuhler and Deitrich, 1984; Lopez-Aumatell et al., 2008, 2009a,b, 2011; Johannesson et al., 2009; Vicens-Costa et al., 2011; Diaz-Moran et al., 2012, 2013a,b,c, 2014; Baud et al., 2013, 2014a,b). Moreover, NIH-HS rats have been shown to

closely resemble RLA-I rats in their coping style and stress sensitivity profiles (e.g., Lopez-Aumatell et al., 2009a; Diaz-Moran et al., 2012, 2013c; Estanislau et al., 2013). Remarkably, regarding schizophrenia-like phenotypes such as PPI and spatial working memory, the scores of the NIH-HS rat stock are very close to those from the RLA-I strain (Oliveras et al., 2015). Interestingly, when these NIH-HS rats are divided for their extreme values of PPI, the rats with a poorer PPI performance also exhibit working memory deficits and latent inhibition while the NIH-HS rats with a better PPI performance have an improved working memory and latent inhibition performance (Oliveras et al., 2015; Sanchez-Gonzalez et al., 2016)

The aim of the present study is to elucidate whether the differences between the Roman strains regarding the HT1AR and HT2AR appear also between NIH-HS rats selected by high or low PPI scores. In this regard, as the previous literature suggests that schizophrenia patients have increased levels of 5-HT1A receptor, and since Klein et al. (2014) found that RHA-I rats displayed increased binding levels of 5-HT1A and 5-HT2A receptors in the PFC, we hypothesized that the NIH-HS rats selected for their poor PPI performance (see "Methods") would exhibit increased expression of these receptors in the PFC. We also carried out an association study in order to investigate if there is any relationship between gene expression and PPI performance in the whole rat sample used.

2. Methods

2.1 Animals

From a pool of rats that were submitted to the PPI test (see below), we used 10 males from the Roman High- (RHA-I) and 10 males from the Roman Low-Avoidance (RLA-I) rat strains (4 months old and 350–450 g), as well as 20 males from the genetically heterogeneous NIH-HS rat stock (NIH-HS: "National Institutes of Health Genetically Heterogeneous Rat Stock, 4 months old and 320–420 g**)** which had been selected for their extreme values on the PPI test (high-PPI NIH-HS group, n=10, and low-PPI NIH-HS group, n=10). The Roman rats came from 7 litters in each group (1-2 animals per litter) while the NIH-HS rats came from 18 litters (1-2 animals per litter). The brains of these rats were removed 3 weeks after the testing session was finished. The prefrontal cortex (PFC), hippocampus (HPC) and striatum (STR) were dissected out bilaterally by free-hand razor dissection and transferred to RNase-

free tubes that were freezed with liquid nitrogen and then stored at -80ºC until further analysis.

2.2 Prepulse inhibition

Four startle boxes (SR-Lab Startle Response System, San Diego Inst., San Diego, USA) were used. They consist in a Plexiglas cylinder situated on the top of a platform with a sensor that detects changes in strength made by the movements of the rat in each trial. Auditory stimuli were delivered by two speakers situated 15 cm from each side of the cylinder. Each box is constantly lit by a 10 W lamp. The data are transduced by an accelerometer into a voltage and then saved into a computer for further analysis.

The startle session started with a 5 min habituation period in the startle chambers. Then, 10 "pulse-alone" trials (105 dB, 40 ms) are delivered in order to obtain a baseline measure of the startle response (Baseline 1). After this, each one of the 6 different types of trials were randomly administered 10 times (60 trials in total): Pulse-alone trials (105 dB, 40 ms, Baseline 2, this was the variable used to calculate the %PPI; see the equation below); Prepulses of 65 dB, 70 dB, 75 dB and 80 dB (20 ms) followed by the pulse (105 dB, 40 ms), with an inter-stimulus interval of 100 ms; finally, 10 trials in which no stimulus was delivered and only the background noise was present (55 dB). At the end of the session, 5 "pulse-alone" trials were delivered to have a measure of habituation to the startle stimulus (Baseline 3). The interval between trials was 10–20 s with a mean of 15s. The maximal magnitude (i.e. the peak) of startle response was recorded during 200 ms after the onset of the pulse.

The percentage of PPI (%PPI) is calculated according to the formula: %PPI = 100 − ((startle amplitude on prepulse trials/startle amplitude on pulse trials) × 100)

2.3 Reverse transcription quantitative polymerase chain reaction (RT-qPCR) of HT1AR, HT2AR in the prefrontal cortex (PFC), hippocampus (HPC) and the striatum (STR)

RNA was extracted from approximately 30 mg of frozen tissue using the NucleoSpin RNA/protein purification kit (MACHEREY-NAGEL, Düren, Germany) according to manufacturer's instructions. Prior RNA extraction frozen brain tissues were homogenized using the MagNA lyser instrument (3000 rpm, 30 s) and related MagNA lyser green beads

(Roche Molecular Biochemicals, Indianapolis, IN, USA) in 1 mL lysis buffer supplemented with the RNA isolation kit. The lysis buffer creates appropriate binding conditions, which favor adsorption of RNA to the silica membrane, and enables protein to pass the specially treated NucleoSpin® RNA/protein column.

All RNA samples were treated once with DNase using TURBO DNA-free Kit (Ambion) according to the manufacturer's instructions. RNA samples were suspended in RNase-free water and were quantified using Agilent 2100 Bioanalyzer (Agilent Technologies). Only samples with RIN ≥ 6 were included in the analysis. RNA samples were stored at -80°C until further use.

To check RNA samples for contamination with nuclear DNA a negative control PCR was done on all samples prior to cDNA synthesis omitting the reverse transcription step.

A two-step real-time PCR was subsequently performed: RNA samples matched on concentration were reverse transcribed into cDNA with qScript cDNA SuperMix Kit (Quanta BioSciences) according to manufacturer's instructions. SuperMix reaction mixture consisted of 5x reaction buffer containing optimized concentrations of $MgCl_2$, dNTPs (dATP, dCTP, dGTP, and dTTP), recombinant RNase inhibitor protein, qScript reverse transcriptase, random primers, oligo(dT) primers, and stabilizers. cDNA synthesis step was performed following incubation times of 5 minutes at 25°C, 30 minutes at 42°C, 5 minutes at 85°C, and 5 minutes at 25°C. Thereafter cDNA product was diluted 5-fold in nuclease-free water and kept at -20°C until further use.

Each qPCR reaction were run in duplicates in 96 well plates with the amounts of forward, backward primers, Fast SYBR® Green Master Mix (Applied Biosystems) and RNase free water according to the manufacturer guidelines. The reactions were run on the Stratagene Mx3005P PCR system (Applied Biosystems) with the following program: one cycle with 20 seconds at 95 ºC (activation), and 45 cycles at 95 ºC for 5 seconds and 60 ºC (denature) for 30 seconds. At the end of the 45th cycle a melting curve program was run: 1 minute 95ºC, 30 s 60ºC and 95 ºC for 30 s. All reactions were run in duplicates and median cycles to threshold (Ct) values were used for analysis.

A comparative cycle of threshold fluorescence (Ct) method was used and the relative transcription level of the target genes (median of the Cts of the duplicates) were normalized to that of the housekeeping genes (glyceraldehyde-3-phosphate dehydrogenase; GAPDH) and

Ribosomal protein L13(Rpl13), and expressed as relative quantity to the calibrator sample using the Pfaffl method (Pfaffl, 2001).

2.4 Statistical analysis

Statistical analysis was performed using the "Statistical Package for Social Science" (SPSS, version 17). PPI testing and PCR experiments were carried out in a counterbalanced manner for the 4 rat groups, but because our hypotheses were focused on RHA-I vs RLA-I and high-PPI NIH-HS vs low-PPI NIH-HS differences (and not in comparing Roman vs NIH-HS rats), pair-wise comparisons were performed using Student's *t*-tests between the two Roman groups and, separately, between the two NIH-HS groups. In order to assess the hypothesis that NIH-HS expression of the serotonin receptors in the PFC will follow the same pattern as the Roman rats (i.e. low-PPI NIH-HS rats were expected to have higher levels of both receptors than high-PPI NIH-HS rats), significance of the student's t-tests were taken as one-tailed $p \leq 0.05$.

Before the analyses some samples from each group were excluded from further analysis as they showed significance in a Grubbs outlier test (the final "n" for each group can be seen in the Figure 1 legend). All the main variables were checked for normality (Kolmogorov-Smirnov). Expression ratios were "ln" transformed in order to achieve normality.

Correlation analysis was performed using Pearson`s correlation coefficients among the main variables of PPI and the gene expression ratios.

Linear discriminant analysis (LDA) was carried out in order to investigate whether the level of expression of the serotonin receptors in the PFC was able to classify the rats in two groups according to their expected performance in the PPI: low scores of PPI : RHA-I rats and low-PPI NIH-HS rats; high scores in the PPI: RLA-I and high-PPI NIH-HS rats. In the first analysis both variables were entered in the discriminant function. Subsequently an analysis with stepwise method with the same variables was done to see if one of the variables had a higher discriminant power. Statistical significance was set at $p \leq 0.05$.

3. Results

Student's t-tests on the expression of each gene in the PFC of the Roman rats showed significant differences between the strains for the *HTR1A* and *HTR2A* ($t(11) = 3.50$ $p = 0.005$ and $t(6.87) = 3.52$ $p = 0.010$, respectively; Fig.1A), indicating that RHA-I rats have augmented expression of both serotonin receptors in the PFC. The same analysis for the NIH-HS groups revealed a significant difference between groups for the *HTR1A* gene ($t(8) = -2.26$ $p = 0.029$, one-tailed, Fig 1A), indicating increased *HTR1A* expression in the low-PPI group.

The same analyses for the expression of the genes in the HPC did not reveal any significant difference between groups. On the other hand, in the STR, student's t-test for the Roman rats revealed a statistically significant difference between strains for the HTR2A gene ($t(3.41) = 3.57$ $p = 0.030$, Fig. 1C), indicating the RHA-I rats exhibit enhanced expression of this gene.

We also conducted a correlational analysis (Pearson's R) that revealed the expected high correlations among PPI variables, and also that there is a positive correlation between the expression of both serotonin receptors within the three areas explored ($Rs > 0.66$ and $p < 0.004$). Remarkably there were significant negative correlations between the *HTR1A* gene expression in the PFC and the performance of the rats in the PPI. Specifically, there were significant correlations between the performance in 70 dB prepulse ($R = -0.54$ $p = 0.048$). Noticeably, the overall performance of the PPI session also correlated negatively with the expression of 5-HT1AR in the PFC ($R= -0.42$ $p = 0.047$). Thus, indicating that a better performance in the PPI is associated with lower levels of the 5-HT1AR in the PFC. Additionally, the expression of *HTR2A* in the PFC was also negatively correlated with the PPI performance in the 70 dB prepulse intensity ($R = -0.41$ $p = 0.045$)

We carried out a linear discriminant analysis (LDA) that included both *HTR1A* and *HTR2A* expression in the PFC. The results of this analysis showed that discriminant function coefficients were 0.89 for the *HTR1A* gene and 0.16 for the *HTR2A* gene, indicating that *HTR1A* gene expression in the PFC had a greater discriminant power . The function accounted for the 35 % of the between group variability (canonical $R^2 = 0.35$; $\lambda = 0.65$, $\chi^2= 8.63$, $p = 0.013$). The structure matrix showed that both variables were highly and positively correlated with the discriminant function, as the loadings were 0.99 for the *HTR1A* and 0.75 for the *HTR2A*. The cross-validated classification revealed that the discriminant function could classify correctly the 87% of the cases.

We conducted the same analysis but with a stepwise method. In this case the only significant factor was *HTR1A* expression. The results are very similar to those obtained when both predictors were entered in the function (canonical $R^2 = 0.35$; $\lambda = 0.65$, $\chi^2 = 8.74$, $p = 0.003$) indicating that *HTR1A* expression alone could classify correctly 82.6% of all the cases.

The results regarding *HTR1A* gene expression in the PFC reported in the LDA, together with the student's t-test comparing the Roman rats, and the significant t-test comparing the NIH-HS groups and the significant correlation ($R = -0.42$ and $R^2 = 0.18$) between the 5-HT1AR expression and the PPI performance, suggest that *HTR1A* gene expression is related to sensorimotor gating in rats.

4. Discussion

The present study confirms that the Roman rats exhibit differences regarding 5-HT1AR and 5-HT2AR regulation in the prefrontal cortex (PFC). The results reveal that RHA-I rats subjected to PPI exhibit enhanced expression of both receptor genes in the PFC. Besides assessing receptor gene expression differences between the Roman strains that could parallel their divergent PPI performance, an important objective of the present study was to evaluate whether these (expected) strain-related divergences could generalize to heterogeneous (outbred) rats selected for their extreme (high or low) PPI scores and whether there could be associations between gene expression and PPI. Remarkably, we found that heterogeneous NIH-HS rats selected for their extremely poor scores of %PPI show enhanced expression of the *HTR1A* gene in the PFC, which is in line with the differences found between the Roman strains. In accordance with these findings, in the whole rat sample we also found, (i) a negative association between *HTR1A* gene expression in the PFC and both %PPI at the 70 dB prepulse intensity and total %PPI, (ii) a negative correlation between *HTR2A* gene expression and %PPI performance at the 70 dB prepulse intensity, and (iii) that discriminant analysis shows that *HTR1A* expression in the PFC by itself classifies 82.6% of the rats according to their PPI performance. The correlational study also shows positive associations between both serotonin genes, which is in line with the notion that the link between these receptors is functionally important regarding cognitive and attentional mechanisms (Aznar and Hervig, 2016).

5-HT1A receptors have been implicated in the cognitive/affective anomalies present in schizophrenia (e.g. Aznar and Hervig 2016; Ohno 2011), and recent clinical studies have shown that atypical antipsychotics have a partial agonism action on that receptor (thus actually leading to a decrease of its function), such as tandospirone and lurasidone, ameliorating the cognitive impairments in schizophrenia patients as well as in animal models (Sumiyoshi et al., 2001, 2007; Nakamura et al., 2009; for review see Ohno 2011). It has been proposed that postsynaptic 5-HT1A receptors tonically inhibit cholinergic and glutamatergic neuronal activity in septo-hippocampal/cortical areas, so that the cognitive improvements produced by 5-HT1AR partial agonists would be due to a disinhibition of these cholinergic/glutamatergic neurons or systems (e.g. Ohno 2011). In any case, the present findings seem to be in line with the above evidence, as we show that rats with relative PPI deficits (i.e. RHA-I and the selected "low PPI NIH-HS" rats), which are known to also display cognitive deficits (Oliveras et al., 2015), exhibit increased *HTR1A* expression in PFC. These negative *HTR1A*-PPI relationship, as well as the negative associations shown by the present correlational and discriminant analyses, seem to be coherent with previous results showing enhanced 5-HT1AR in the PFC of schizophrenic patients (Meltzer and Sumiyoshi, 2008; Selvaraj et al., 2014; Abi-Dargham, 2007; Burnet et al., 1996; Joyce et al., 1993), that usually show PPI impairments. In a related vein, Richtand et al. (2007) have suggested that an increased binding affinity of the 5-HT1A receptor is associated with a poorer performance of atypical antipsychotics, which could cohere with our finding that clozapine was unable to rescue the PPI deficits exhibited by RHA-I rats, while haloperidol was able to ameliorate them (Oliveras et al., 2017).

On the other hand, there are studies showing either enhanced or decreased 5-HT2A binding in the PFC of schizophrenic patients in the absence of mRNA changes (for review see Baou et al., 2016). A reasonable explanation for these controversial results is that most of the studies that have found decreased *HTR2A* expression or diminished 5-HT2AR binding levels in PFC have been carried out in patients treated chronically with atypical antipsychotics, which could decrease 5-HT2AR binding levels (Muguruza et al., 2013; remind that the therapeutic effects of atypical antipsychotics on these patients are partially due to their antagonism of 5-HT2A receptors). In fact, increased binding of the 5-HT2AR in PFC has been found in untreated (antipsychotic-free) schizophrenic patients (Muguruza et al., 2013; Gonzalez-Maeso et al., 2008).

The negative correlation between *HTR2A* gene expression in the PFC and PPI (at 70dB) and the fact that RHA-I rats show enhanced *HTR2A* expression in PFC, are also in line with the evidence that 5-HT2AR agonists like DOI and LSD, which impair PPI, produce their hallucinogenic activity through their action on that receptor, and that 5-HT2AR binding is increased in PFC from schizophrenic patients (Gonzalez-Maeso et al., 2008; Muguruza et al., 2013). Nevertheless, *HTR2A* expression in the PFC (nor in any other area) was not different between high and low PPI NIH-HS rats, which limit the generalizability of the results. Current studies are being devoted to evaluate the relationship between PPI levels and *HTR2A* and *HTR1A* gene expression in larger samples of genetically heterogeneous NIH-HS rats.

The present gene expression results from the Roman strains are in accordance with the report by Klein et al. (2014) showing that RHA-I rats exhibit enhanced 5-HT1AR and 5-HT2AR binding in the prefrontal cortex (PFC) compared to the RLA-I rats, where 5-HT2AR binding was correlated with impulsivity in the 5-choice serial reaction time task (5-CSRTT) in RHA-I rats. However, in a later study by Fomsgaard et al (2017) we did not find an association between the increased receptor binding in PFC in the RHA-I and an increase in gene expression of the 5-HT2AR. This suggest that the different receptor pools (membrane bound, intracellular protein reservoir, and transcribed mRNA) are regulated independently, and that *HTR2A* and *HTR1A* gene expression may be a direct response to the PPI test. Indeed, *HTR2A* cortical expression responds rapidly to environmental stimuli (Maple et al., 2015). Thus, the differential 5-HT1AR and 5-HT2AR regulation, together with the reported hyperdopaminergic state in the striatum and nucleus accumbens of RHA rats (Tournier et al., 2013; Giorgi et al., 2007; Guitart-Masip et al., 2008a-b), appear to cohere with their profile of schizophrenia-relevant symptoms (Del Rio et al., 2014; Oliveras et al., 2015, 2016, 2017), impulsiveness (which is linked to increased 5-HT2AR; e.g. Lambe et al., 2011, Aznar and Hervig, 2016; Klein et al., 2014) and decreased anxiety (which is related to increased 5-HT1AR; e.g. Lambe et al., 2011).

The correlational study shows, as expected, that expression of both serotonin receptors is positively correlated within the two regions.. The interaction between both receptors is functionally important. For instance in regulating gamma oscillations triggered by tasks that assess cognitive processes (Williams and Boksa, 2010 Uhlhaas and Singer, 2010). The discriminant analysis conducted shows that *HTR1A* expression in the PFC is more robustly associated to PPI than *HT2AR* expression, whichtogether with the finding that both RHA-I and low-PPI NIH-HS rats (both groups displaying poor PPI) show enhanced expression of the

former gene, may suggest that 5-HT1AR expression (or/and binding) might constitute a biomarker of sensorimotor gating deficits (and hence of some schizophrenia-relevant symptoms).

In conclusion, the differences found between both Roman rat strains as well as between the selected low-PPI and high-PPI NIH-HS rats, and the correlation coefficients and discriminant analysis suggest that both serotonin receptors are negatively associated with sensorimotor gating (PPI), a pre-attentional process that is affected in schizophrenia. The results found in genetically heterogeneous NIH-HS rats suggest that cortical *HTR1A* gene expression is the most sensible to the phenotypic selection for high or low PPI, which adds support to the generalizability of the findings from the Roman strains. This convergence of findings might suggest that this gene (*HTR1A*) is more clearly involved in PPI performance (and hence in sensorimotor gating) than the *HTR2A* gene, but this issue has to be further addressed in studies involving larger heterogeneous (i.e. NIH-HS) rat samples and/or different rat strains.

References

Abi-Dargham A (2007) Alterations of serotonin transmission in schizophrenia. *Int Rev Neurobiol* 78:133–164.

Arranz M, Collier D, Sodhi M, Ball D, Roberts G, Price J, Sham P, Kerwin R (1995) Association between clozapine response and allelic variation in 5-HT2A receptor gene. *Lancet* 346:281–282

Arranz MJ, Bolonna AA, Munro J, Curtis CJ, Collier DA, Kerwin RW (2000) The serotonin transporter and clozapine response. *Mol Psychiatry* 5:124–125.

Aznar S, Hervig ME (2016) The 5-HT_{2A} serotonin receptor in executive function: implications for neuropsychiatric and neurodegenerative diseases. *Neurosci & Biobehav Rev* 64: 63–82.

Baou M, Boumba VA, Petrikis P, Rallis G, Vougiouklakis T, Mavreas V (2016) A review of genetic alterations in the serotonin pathway and their correlation with psychotic diseases and response to atypical antipsychotics. *Schizophr Res* 170:18–29.

Baud A, Flint J, Fernández-Teruel A, and The Rat Genome Sequencing Mapping Consortium (2014b) Identification of genetic variants underlying anxiety and multiple sclerosis in heterogeneous stock rats. *World J Neurosci* 4:216–224

Baud A, Guryev V, Hummel, O, Johannesson M, The Rat Genome Sequencing Mapping Consortium and Flint J (2014a) Genomes and phenomes of a population of outbred rats and its progenitors. *Sci Data* 1:140011.

Baud A, Hermsen R, Guryev V, Stridh P, Graham D, McBride MW et al. (2013) Combined sequence-based and genetic mapping analysis of complex traits in outbred rats. *Nat Genet* 45:767–775.

Burnet PW, Eastwood SL, Harrison PJ (1996) 5-HT_{1A} and 5-HT_{2A} receptor mRNAs and binding site densities are differentially altered in schizophrenia. *Neuropsychopharmacology* 15:442–455

del Río C, Oliveras I, Cañete T, Blázquez G, Tobeña A and Fernández-Teruel A (2014) Genetic Rat Models of Schizophrenia-Relevant Symptoms, *World J Neurosci* (June), 261–278.

Díaz-Morán S, Estanislau C, Cañete T, Blázquez G, Ráez A, Tobeña A, and Fernández-Teruel A (2014) Relationships of open-field behaviour with anxiety in the elevated zero-maze test: focus on freezing and grooming. *World J Neurosci* 4:1–11.

Díaz-Morán S, Martínez-Membrives E, López-Aumatell R, Cañete T, Blázquez G, Palencia M, et al. (2013c) What can we learn on rodent fearfulness/anxiety from the genetically heterogeneous NIH-HS rat stock? *Open J Psychiatry*. 3:238–250

Díaz-Morán S, Palència M, Mont-Cardona C, Cañete T, Blázquez G, Martínez-Membrives E, et al. (2012) Coping style and stress hormone responses in genetically heterogeneous rats: comparison with the Roman rat strains. *Behav Brain Res* 228: 203–210.

Díaz-Morán S, Palència M, Mont-Cardona C, Cañete T, Blázquez G, Martínez-Membrives E, et al. (2013a) Gene expression in hippocampus as a function of differential trait anxiety levels in genetically heterogeneous NIH-HS rats. *Behav Brain Res* 257:129–139.

Díaz-Morán S, Palència M, Mont-Cardona C, Cañete T, Blázquez G, Martínez-Membrives E, et al. (2013b) Gene expression in amygdala as a function of differential trait anxiety levels in genetically heterogeneous NIH-HS rats. *Behav Brain Res* 252: 422–431.

Driscoll P, Escorihuela R M, Fernández-Teruel A, Giorgi O, Schwegler H, Steimer T, et al. (1998) Genetic selection and differential stress responses. The Roman lines/strains of rats. Ann. N.Y. Acad. Sci. 851, 501–510.

Driscoll P, Fernández-Teruel A, Corda MG, Giorgi O, Steimer T (2009) Some guidelines for defining personality differences in rats, in Handbook of Behavior Genetics, ed Yong-Kyu K., editor. (New York, NY: Springer), 281–300.

Erritzoe D, Rasmussen H, Kristiansen KT, Frokjaer VG, Haugbol S, Pinborg L, et al.(2008) Cortical and subcortical 5-HT2A receptor binding in neuroleptic-naïve first-episode schizophrenic patients. *Neuropsychopharmacology* 33:2435–41.

Escorihuela RM, Fernández-Teruel A, Gil L, Aguilar R, Tobeña A and Driscoll P (1999) Inbred Roman high- and low-avoidance rats: differences in anxiety, novelty-seeking, and shuttlebox behaviors. *Physiol Behav* 67:19–26.

Esnal A, Sánchez-González A, Río-Álamos C, Oliveras I, Cañete T, Blázquez G, et al. (2016) Prepulse inhibition and latent inhibition deficits in Roman high-avoidance vs. Roman low-avoidance rats: Modeling schizophrenia-related features. *Physiol Behav* 163:267–273.

Estanislau C, Díaz-Morán S, Cañete T, Blázquez G, Tobeña A and Fernández-Teruel A (2013) Context-dependent differences in grooming behavior among the NIH heterogeneous stock and the Roman high- and low-avoidance rats. *Neurosci Res* 77: 187–201.

Fernández-Teruel A, Blázquez G, Pérez M, Aguilar R, Cañete T, Guitart M et al. (2006) Latent inhibition threshold in roman high-avoidance rats: a psychogenetic model of abnormalities in attentional filter? *Actas Esp Psiquiatr* 34:257–263.

Fomsgaard L, Moreno J L, de la Fuente Revenga M, Brudek T, Adamsen D, Río-Álamos C, et al. (2017). Differences in 5-HT2A and mGlu2 Receptor Expression Levels and Repressive Epigenetic Modifications at the 5-HT2A Promoter Region in the Roman Low- (RLA-I) and High- (RHA-I) Avoidance Rat Strains. Mol Neurobiol doi:10.1007/s12035-017-0457-y

Geyer M A, Krebs-Thomson K, Braff D L, Swerdlow N R (2001) Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology* 156:117–154.

Geyer MA and Vollenweider FX (2008) Serotonin research: contributions to understanding psychoses. *Trends Pharmacol Sci* 29: 445–453.

Giorgi O, Piras G, Corda MG (2007) The psychogenetically selected Roman high- and low-avoidance rat lines: a model to study the individual vulnerability to drug addiction. *Neurosci Biobehav Rev* 31:148–163.

González-Maeso J, Ang RL, Yuen T, Chan P, Weisstaub NV, López-Giménez JF et al. (2008) Identification of a serotonin/glutamate receptor complex implicated in psychosis. *Nature 452*:93–7.

Guitart-Masip M, Johansson B, Fernández-Teruel A, Tobeña A, Giménez-Llort L (2008) Divergent effect of the selective D3 receptor agonist PD-128,907 on locomotor activity in Roman high- and low-avoidance rats: relationship to NGFI-A gene expression in the Calleja islands. *Psychopharmacology* 196:39–49.

Hansen C and Spuhler K (1984) Development of the national institutes of health genetically heterogeneous stock. *Alcohol Clin Exp Res* 8:477–479.

Johannesson M, Lopez-Aumatell R, Stridh P, Diez M, Tuncel J, Blázquez G et al. (2009) A resource for the simultaneous high-resolution mapping of multiple quantitative trait loci in rats: the NIH heterogeneous stock. *Genome Res* 19:150–158.

Joyce JN, A. Shane A, Lexow N, Winokur A, Casanova, MF Kleinman JE (1993) Serotonin uptake sites and serotonin receptors are altered in the limbic system of schizophrenics. *Neuropsychopharmacology* 8:315-336

Klein AB, Ultved L, Adamsen D, Santini MA, Tobeña A, Fernández-Teruel A et al. (2014) 5-HT(2A) and mGlu2 receptor binding levels are related to differences in impulsive behavior in the Roman Low- (RLA) and High- (RHA) avoidance rat strains. *Neuroscience 263*:36–45.

Kohl S, Heekeren K, Klosterkötter J, and Kuhn J (2013) Prepulse inhibition in psychiatric disorders -apart from schizophrenia. *J Psychiatr Res 47*: 445–52.

Lambe EK, Fillman SG, Webster MJ, Weickert CS (2011) Serotonin receptor expression in human prefrontal cortex: balancing excitation and inhibition across postnatal development. *PLoS One* 6:e22799.

López-Aumatell R, Blázquez G, Gil L, Aguilar R, Cañete T, Giménez-Llort L, et al. (2009c) The Roman High and Low-Avoidance rat strains differ in fear-potentiated startle and classical aversive conditioning. *Psicothema* 21: 27–32.

López-Aumatell R, Guitart-Masip M, Vicens-Costa E, Gimenéz-Llort L, Valdar W, Johannesson M et al. (2008) Fearfulness in a large N/Nih genetically heterogeneous rat stock: differential profiles of timidity and defensive flight in males and females. *Behav Brain Res* 188, 41–55.

López-Aumatell R, Martínez-Membrives E, Vicens-Costa E, Cañete T, Blázquez G, Mont-Cardona C et al. (2011) Effects of environmental and physiological covariates on sex differences in unconditioned and conditioned anxiety and fear in a large sample of genetically heterogeneous (N/Nih-HS) rats. *Behav Brain Funct* 7:48.

López-Aumatell R,Vicens-Costa E,Guitart-Masip M, Martínez-Membrives E, Valdar W, Johannesson M et al. (2009a) Unlearned anxiety predicts learned fear: a comparison among heterogeneous rats and the Roman rat strains. *Behav Brain Res* 202: 92–101.

López-Aumatell R,Vicens-Costa E,Guitart-Masip M, Martínez-Membrives E, Valdar W, Johannesson M et al. (2009b) Ansiedad en ratas genéticamente heterogéneas: hacia la identificación de genes para caracteres conductuales cuantitativos. *Ansiedad Estrés* 15:67–84

Maple AM, Zhao X, Elizalde DI, McBride AK, Gallitano AL (2015) Htr2a expression responds rapidly to environmental stimuli in an Egr3-dependent manner. *ACS Chem. Neurosci* 6:1137–1142.

McCreary A and Newman-Tancredi A (2015) Serotonin 5-HT_{1A} Receptors and Antipsychotics - An Update in Light of New Concepts and Drugs. *Curr Pharm Design 21*:3725–3731.

Meltzer HY and Sumiyoshi T (2008) Does stimulation of 5-HT_{1A} receptors improve cognition in schizophrenia? *Behav Brain Res* 195:98–102.

Muguruza C, Moreno JL, Umali A, Callado LF, Meana JJ, González-Maeso J (2013) Dysregulated 5-HT_{2A} receptor binding in postmortem frontal cortex of schizophrenic subjects.*Eur Neuropsychopharmacol* 23:852–864.

Nakamura M, Ogasa M, Guarino J, et al. (2009) Lurasidone in the treatment of acute schizophrenia: A double-blind, placebo-controlled trial. *J Clin Psychiatry* 70:829–836.

Norton N and Owen MJ (2005) HTR2A: association and expression studies in neuropsychiatric genetics. *Ann Med* 37:121–129

Ohno Y (2011) Therapeutic role of 5-HT1A receptors in the treatment of schizophrenia and Parkinson's disease. *CNS Neurosci Ther* 17:58–65.

Oliveras I, Sánchez-González A, Piludu MA, Gerboles C, Río-Álamos C, Tobeña A, Fernández-Teruel A (2016) Divergent effects of isolation rearing on prepulse inhibition, activity, anxiety and hippocampal-dependent memory in Roman high- and low-avoidance rats: A putative model of schizophrenia-relevant features. *Behav Brain Res* 314:6-15

Oliveras I, Sánchez-González A, Sampedro-Viana D, Piludu MA, Río-Alamos C, Giorgi O, Corda MG, Aznar S, González-Maeso J, Gerbolés C, Blázquez G, Cañete T, Tobeña A, Fernández-Teruel A (2017) Differential effects of antipsychotic and propsychotic drugs on prepulse inhibition and locomotor activity in Roman High- (RHA) and Low-Avoidance (RLA) rats. *Psychopharmacology 234:* 957-975

Oliveras I, Río-Álamos C, Cañete T, Blázquez G, Martínez-Membrives E, Giorgi O et al. (2015) Prepulse inhibition predicts spatial working memory performance in the inbred Roman high- and low-avoidance rats and in genetically heterogeneous NIH-HS rats: relevance for studying pre-attentive and cognitive anomalies in schizophrenia. *Front Behav Neurosci*, *9*:213.

Pfaffl MW (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 29:45.

Richtand NM, Welge JA, Logue AD, et al. (2007) Dopamine and serotonin receptor binding and antipsychotic efficacy. *Neuropsychopharmacology* 32:1715–1726

Río-Álamos C, Oliveras I, Cañete T, Blázquez G, Martínez-Membrives E, Tobeña A, Fernández-Teruel A (2015) Neonatal handling decreases unconditioned anxiety, conditioned fear, and improves two-way avoidance acquisition: a study with the inbred Roman high (RHA-I)- and low-avoidance (RLA-I) rats of both sexes. *Front Behav Neurosci* 9:174.

Río-Álamos C, Oliveras I, Piludu MA, Gerbolés C, Cañete T, Blázquez G, Lope-Piedrafita S, Martínez-Membrives E, Torrubia R, Tobeña A, Fernández-Teruel A (2017) Neonatal handling enduringly decreases anxiety and stress responses and reduces hippocampus and amygdala volume in a genetic model of differential anxiety: Behavioral-volumetric associations in the Roman rat strains. *European Neuropsychopharmacology* 27:146–158

Sánchez-González A, Esnal A, Río-Álamos C, Oliveras I, Cañete T, Blázquez G, et al. (2016) Association between prepulse inhibition of the startle response and latent inhibition of two-way avoidance acquisition: A study with heterogeneous NIH-HS rats. *Physiol Behav* 155:195–201.

Selvaraj S, Arnone D, Cappai A, and Howes O (2014) Alterations in the serotonin system in schizophrenia: A systematic review and meta-analysis of postmortem and molecular imaging studies. *Neurosci & Biobehav Rev* 45:233–245.

Shilling P D, Saint Marie RL, Shoemaker JM and Swerdlow NR (2008) Strain differences in the gating-disruptive effects of apomorphine: relationship to gene expression in nucleus accumbens signaling pathways. *Biol Psychiatry* 63:748–58.

Spuhler K and Deitrich RA (1984) Correlative analysis of ethanol-related phenotypes in rat inbred strains. *Alcohol Clin Exp Res* 8:480–484.

Sumiyoshi T, Matsui M, Nohara S, et al. (2001) Enhancement of cognitive performance in schizophrenia by addition of tandospirone to neuroleptic treatment. *Am J Psychiatry* 158:1722–1725.

Sumiyoshi T, Park S, Jayathilake K, Roy A, Ertugrul A, Meltzer HY (2007) Effect of buspirone, a serotonin$_{1A}$ partial agonist, on cognitive function in schizophrenia: A randomized, double-blind, placebo-controlled study. *Schizophrenia Res* 95:158–168.

Swerdlow NR, Light GA, Trim RS, Breier MR, Hines SR, and Powell SB (2013a) Forebrain gene expression predicts deficits in sensorimotor gating after isolation rearing in male rats. *Behav Brain Res 257*:118–28.

Swerdlow NR, Powell SB, Breier MR, Hines SR, and Light GA (2013b) Coupling of gene expression in medial prefrontal cortex and nucleus accumbens after neonatal ventral hippocampal lesions accompanies deficits in sensorimotor gating and auditory processing in rats. *Neuropharmacology 75*:38–46.

Tournier BB, Steimer T, Millet P, Moulin-Sallanon M, Vallet P, Ibañez V, Ginovart N (2013) Innately low D2 receptor availability is associated with high novelty-seeking and enhanced behavioural sensitization to amphetamine. *Int J Neuropsychopharmacol* 16:1819–1834.

Uhlhaas PJ and Singer W (2010) Abnormal neural oscillations and synchrony in schizophrenia. *Nat Rev Neurosci* 11:100–113

Vicens-Costa E, Martínez-Membrives E, López-Aumatell R, Guitart-Masip M, Cañete T, Blázquez G et al. (2011) Two-way avoidance acquisition is negatively related to conditioned freezing and positively associated with startle reactions: a dissection of anxiety and fear in genetically heterogeneous rats. *Physiol Behav* 103:148–156.

Williams S and Boksa P (2010) Gamma oscillations and schizophrenia. *J Psychiatry Neurosci* 35:75–77.

Wood CM, Nicolas CS, Choi S-L, Roman E, Nylander I, Fernández-Teruel A et al. (2016) Prevalence and influence of cys407* Grm2 mutation in Hannover-derived Wistar rats: mGlu2 receptor loss links to alcohol intake, risk taking and emotional behaviour. *Neuropharmacology*

Tables and Figure legends

Table 1. - Primers used for each gene in the qPCR

Table 2. - Mean (±S.E.M) of the total %PPI of the rats of each group. [a] $p < 0.05$ significant student's t-test between the Roman strains. *** $p < 0.001$ significant student's t-test between the NIH-HS groups (n: 10 for all groups).

Table 3. - Pearson`s correlation coefficients between the gene expression ratios and the main PPI measures. *$p < 0.05$ **$p < 0.01$ (two-tailed).

Figure 1. - A) *HTR1A* and *HTR2A* expression in the PFC of the RHA-I (n= 7), RLA-I (n= 6), low-PPI NIH-HS (n= 6) and High-PPI NIH-HS (n= 4 and 5, respectively). B) *HTR1A* and *HTR2A* expression in the HPC of the RHA-I (n= 8), RLA-I (n= 9),low-PPI NIH-HS (n= 9 and 8, respectively) and high-PPI NIH-HS (n=9). C) *HTR1A* and *HTR2A* expression in the STR of the RHA-I (n=7 and 4, respectively), RLA-I (n= 6 and 4 respectively), low-PPI NIH-HS (n= 5) and High-PPI NIH-HS (n= 7 and 6, respectively). ** $p < 0.01$ * $p < 0.05$ between the groups indicated (student's t-test, two-tailed), [a] $p<0.05$ between the groups indicated (student's t-test, one-tailed).

Tables and Figures

Table 1

Gene	Primer sequence	Concentration	Annealing /extension
GAPDH	Forward: 5'-CAT CAA GAA GGT GAA GCA-3'	300 nM	60ºC+72ºC
	Reverse: 5'-CTG TTG AAG TCA CAG GAG ACA-3'		
Rpl13	Forward: 5'-AGC AGC TCT TGA GGC TAA GG-3'	300 nM	60ºC+72ºC
	Reverse: 5'-GGG TTC ACA CCAAGA GTC CA-3'		
HTR1A	Forward: 5'-ATC AGC AAG GAC CAC GGC TAC A-3'	500 nM	62ºC+82ºC
	Reverse: 5'-TGT CCG TTC AGG CTC TTC TTG G -3'		
HTR2A	Forward: 5'-CCA CAG CCG CTT CAA CTC-3'	300 nM	58ºC+76ºC
	Reverse: 5'-GCA GCT CCC CTC CTT AAA GA-3'		

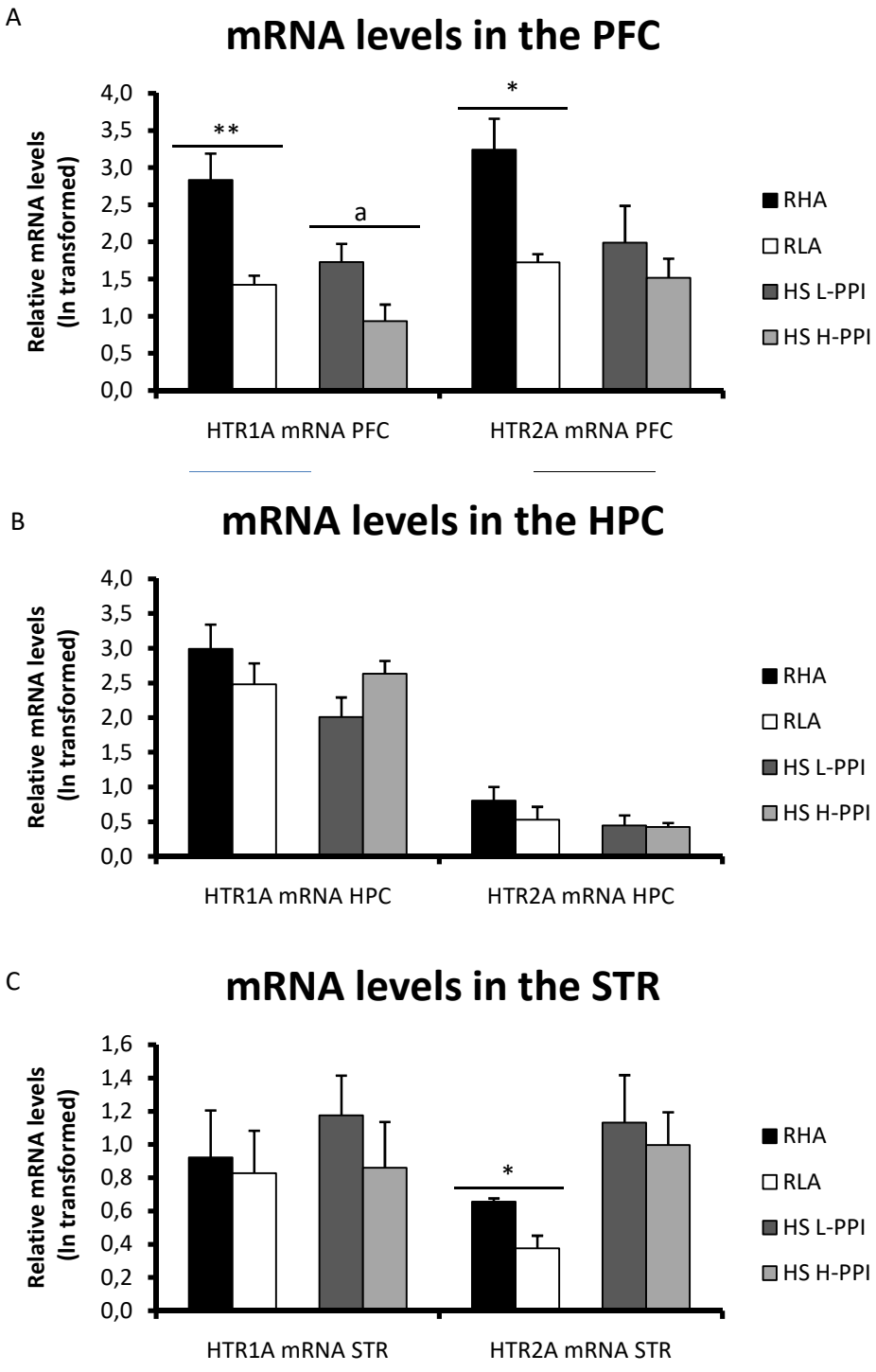
Table 2

STRAIN	%PPI
RHA-I	43.61 (7.32)[a]
RLA-I	63.92 (3.73)[a]
NIH-HS high-PPI	83.70 (1.28)***
NIH-HS low-PPI	38.12 (4.41)***

Table 3

	1	2	3	4	5	6	7	8	9	10	11
1.-HTR1A PFC	1										
2.-HTR2A PFC	**0.75****	1									
3.-HTR1A HPC	-0.09	0.24	1								
4.-HTR2A HPC	-0.04	-0.12	**0.66****	1							
5.-HTR1A STR	-0.17	-0.02	-0.24	-0.26	1						
6.-HTR2A STR	-0.20	-0.29	-0.12	-0.31	**0.68****	1					
7.- %PPI 65	-0.29	-0.20	-0.01	-0.12	0.13	-0.11	1				
8.- %PPI 70	**-0.54***	**-0.41***	0.18	0.05	0.12	-0.00	**0.66****	1			
9.- %PPI 75	-0.17	-0.10	0.14	-0.04	0.10	-0.12	**0.89****	**0.65****	1		
10.- %PPI 80	-0.35	-0.30	0.01	-0.05	0.13	0.14	**0.76****	**0.76****	**0.78****	1	
11.- PPI MEAN	**-0.42***	-0.31	0.11	-0.04	0.13	-0.03	**0.92****	**0.88****	**0.91****	**0.90****	1

Figure 1


A
mRNA levels in the PFC
Relative mRNA levels (ln transformed)
4,0
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
**
a
*
HTR1A mRNA PFC
HTR2A mRNA PFC
RHA
RLA
HS L-PPI
HS H-PPI
B
mRNA levels in the HPC
Relative mRNA levels (ln transformed)
HTR1A mRNA HPC
HTR2A mRNA HPC
RHA
RLA
HS L-PPI
HS H-PPI
C
mRNA levels in the STR
Relative mRNA levels (ln transformed)
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
*
HTR1A mRNA STR
HTR2A mRNA STR
RHA
RLA
HS L-PPI
HS H-PPI

20. Annex 2:

Copyright Springer

Articles disseminated via http://link.springer.com are indexed, abstracted and referenced by many abstracting and information services, bibliographic networks, subscription agencies, library networks, and consortia.

After submission of the agreement signed by the corresponding author, changes of authorship or in the order of the authors listed will not be accepted by Springer.