TREBALL 4

Activation of piramidal cells in rat medial prefrontal cortex projecting to ventral tegmental area by a 5-HT$_{1A}$ receptor agonist.

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En aquest article mostrem que l'administració sistèmica de l'agonista del receptor de serotonina 5-HT$_{1A}$ BAY x 3702 incrementa l'activitat de les neurones piramidals de l'escorça prefrontal medial que projecten a l'àrea tegmental ventral.

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Activation of pyramidal cells in rat medial prefrontal cortex projecting to ventral tegmental area by a 5-HT$_{1A}$ receptor agonist

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Abstract

5-HT$_{1A}$ receptor agonists increase the activity of dopamine (DA) neurons in the ventral tegmental area (VTA) and DA release in medial prefrontal cortex (mPFC). The mPFC is enriched in 5-HT$_{1A}$ receptors and projects to the VTA, where mesocortical dopaminergic neurons originate. We examined whether 5-HT$_{1A}$ receptor activation can modulate the activity of mPFC pyramidal neurons projecting to VTA. These were identified by antidromic stimulation from the VTA and were recorded extracellularly in anesthetized rats. The selective 5-HT$_{1A}$ agonist BAY $\times$ 3702 (10–80 µg/kg i.v.) increased the firing rate in 14/19 neurons (283±79%) and reduced the activity of 5/19 neurons (22±11%), resulting in an overall 2.2-fold increase of the firing rate. Both effects were blocked by the selective 5-HT$_{1A}$ antagonist WAY-100635. These results suggest that the increase in dopaminergic activity produced by 5-HT$_{1A}$ receptor activation can be driven by an increase in the activity of projection neurons in mPFC.

1. Introduction

The mesolimbic and mesocortical dopamine (DA) systems arising from the ventral tegmental area (VTA) are deeply involved in a large number of brain functions, including cognition, memory, reward and behavioural control (Glowinski et al., 1984; Williams and Goldman-Rakic, 1995; Robbins, 2000; Tzschentke and Schmidt, 2000; Schultz, 2004). Derangements of these ascending systems likely occur in schizophrenia (Carlsson, 1988; Weinberger et al., 1994; Laruelle et al., 1996; Abi-Dargham et al., 2001), a disease whose classical treatment is based on the blockade of DA actions on D2 receptors (Seeman and Lee, 1975). However, more recent drugs (atypical anti-psychotics) display a low affinity for D2 receptors and preferentially occupy serotonergic 5-HT$_{2A}$ receptors in brain

(Nyberg et al., 1998) for which they possess a high in vitro affinity (Meltzer, 1999).

Interestingly, atypical (but not conventional) antipsy-chotics increase DA release in medial prefrontal cortex (mPFC) by a mechanism dependent on the activation of 5-HT$_{1A}$ receptors (Rollema et al., 1997, 2000; Kuroki et al., 1999; Rollema et al., 2000; Ichikawa et al., 2001), despite this they display negligible in vitro affinity for such receptors (Newman-Tancredi et al., 2003) (see however Chou et al., 2003). This has raised the interest in 5-HT$_{1A}$ receptors as potential targets for new antipsychotic drugs (Millan, 2000; Bantick et al., 2001). In parallel with these effects of atypical antipsychotics, presumably mediated by indirect 5-HT$_{1A}$ receptor activation, the systemic adminis-tration of selective 5-HT$_{1A}$ receptor agonists increases DA neuron activity and DA release in prefrontal cortex (PFC) (Arborelius et al., 1993; Lejeune and Millan, 1998, 2000; Sakaue et al., 2000).

The activity of mesocortical DA neurons is controlled, among other areas, by the mPFC, which projects to VTA (Thierry et al., 1979, 1983; Tong et al., 1996, 1998; Carr and

Sesack, 2000a,b). Additionally, the PFC may control the activity of VTA neurons indirectly, through the pedunculo-pontine tegmental nucleus (glutamatergic/cholinergic inputs) and the nucleus accumbens-ventral pallidum pathway (GABA inputs), among others (Tzschenke and Schmidt, 2000; Sesack et al., 2003; Adell and Artigas, 2004). These inputs control, respectively, phasic and tonic changes in the activity of DA neurons (Floresco et al., 2003).

Recent data from this laboratory indicate that 50–60% of the pyramidal neurons and 20–30% of the GABAergic interneurons in mPFC express 5-HT$_{1A}$ receptors (Santana et al., 2004) in close overlap with projection neurons to VTA (Thierry et al., 1979, 1983; Sesack et al., 2003). Based on these results and on previous evidence suggesting a 5-HT$_{1A}$ modulation of dopaminergic neurons (see above), we conducted the present study under the working hypothesis that the activation of 5-HT$_{1A}$ receptors may alter the activity of prefrontal inputs to midbrain, resulting in a subsequent change in dopaminergic activity. Hence, we examined the actions of the highly selective and potent 5-HT$_{1A}$ receptor agonist BAY $\times$ 3702 on the activity of pyramidal neurons in the medial PFC (mPFC) projecting to the VTA.

2. Experimental procedures

2.1. Animals

Male albino Wistar rats (230–300 g; Iffa Credo, Lyon, France) were kept in a controlled environment (12 h light–dark cycle and 22±2 °C room temperature) with food and water provided ad libitum. Animal care followed the European Union regulations (O.J. of E.C. L358/1 18/12/1986) and was approved by the Institutional Animal Care and Use Committee. Stereotaxic coordinates (in mm) were taken from bregma and duramater according to the atlas of Paxinos and Watson (1986).

2.2. Drugs

WAY-100635 was from RBI (Natick, MA). BAY $\times$ 3702 (De Vry et al., 1988) was kindly provided by BAYER AG. Concentrated stock solutions were prepared and aliquots were stored at -80 °C. Working solutions were prepared daily by dilution. BAY $\times$ 3702 was administered i.v. at 10–80 µg/kg (free base) and WAY-100635 at the dose of 30–50 µg/kg i.v. Drugs were dissolved in saline at the appropriate concentrations and injected (up to 1 ml/kg) through the femoral vein.

2.3. Single unit recordings

We examined the responses elicited in pyramidal neurons of the mPFC by the systemic administration of BAY $\times$ 3702 in anesthetized rats. Recordings were made essentially as described in Puig et al. (2003). Rats were administered chloral hydrate (400 mg/kg i.p.) and positioned in a David Kopf stereotaxic frame. Additional doses of chloral hydrate (80 mg/kg) were administered i.v. through the femoral vein. Typically, recordings were made between 10 and ~45 min after additional doses of anesthetic to avoid the effects of peak concentrations of chloral hydrate during recordings. Body temperature was maintained at 37 °C throughout the experiment with a heating pad. In order to minimize pulsation, the atlanto-occipital membrane was punctured to release some CSF.

Bipolar stimulating electrodes consisted of two stainless steel enamel-coated wires (California Fine Wire, Grover Beach, CA) with a diameter of 150 µm and a tip separation of ~100 µm and in vitro impedances of 10–30 KΩ. Stimulating electrodes were stereotaxically implanted in the VTA (AP -6.0, L -0.5, DV -8.2). After implant, the electrodes were secured to the skull with glue and dental cement. Constant current electrical stimuli were generated with a Grass stimulation unit S-48 connected to a Grass SIU 5 stimulus isolation unit. Stimulating current was typically between 0.1 and 1.7 mA, 0.2 ms square pulses at 0.9 Hz.

Pyramidal neurons were recorded extracellularly with glass micropipettes pulled from 2.0-mm capillary glass (WPI, Saratosa, FL) on a Narishige PE-2 pipette puller (Narishige Sci. Inst., Tokyo, Japan). Microelectrodes were filled with 2 M NaCl. Typically, in vitro impedance was between 4 and 10 MΩ. Single unit extracellular recordings were amplified with a Neurodata IR283 (Cygnus Technology Inc., Delaware Water Gap, PA), postamplified and filtered with a Cibertec amplifier (Madrid, Spain) and computed on-line using a DAT 1401plus interface system Spike2 software (Cambridge Electronic Design, Cambridge, UK). Descents in mPFC were carried out at AP $+3.2$–3.4, L -0.5 to -1.0, DV -1.0 to -4.0 below the brain surface. We systematically confirmed that only a single pyramidal neuron was recorded by a) identification by antidromic activation from VTA and b) collision extinction with spontaneously occurring spikes (Fuller and Schlag, 1976). Neurons without antidromic activation or without spontaneous firing activity were not considered. After the identification of a pyramidal neuron antidromically activated from the VTA, basal firing activity was recorded for 5 min and then, increasing doses of BAY $\times$ 3702 were administered i.v., followed by WAY-100635 when appropriate.

At the end of the experiments, rats were killed by an overdose of anesthetic. The placement of the stimulating electrodes was verified histologically. Rats were transcardially perfused with saline followed by 10% formalin solution (Sigma). Brains were post-fixed, coronally sectioned (80 µm) and stained with Neutral Red. The data from rats with electrodes implanted outside the VTA were not used.

2.4. Data and statistical analysis

Changes in firing rate were quantified by averaging the values in the second minute after each BAY $\times$ 3702

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159 injection. The effects of BAY × 3702 and WAY-100635
160 were assessed by one-way repeated measures ANOVA. Data
161 are expressed as the mean ± SEM. Statistical significance has
162 been set at the 95% confidence level (two tailed).

3. Results

164   Fig. 1 shows the identification of a pyramidal neuron by
165 the antidromic stimulation from the VTA. A total of 19
166 pyramidal neurons were recorded that projected to the VTA,
167 whose approximate location is shown in Fig. 1. The mean
168 latency of antidromic responses induced by VTA stimula-
169 tion was 9.5 ± 1.2 ms ($n = 19$), a value falling within the
170 range previously reported values for cortical pyramidal
171 neurons projecting to VTA (Thierry et al., 1983; Pirot et al.,
172 1992; Puig et al., 2003).
173   Baseline firing rate of the recorded pyramidal neurons
174 was 3.3 ± 0.7 spikes/s ($n = 19$). The administration of
175 BAY × 3702 exerted two opposite effects on the firing rate

176 of pyramidal neurons. Fourteen of the recorded units were
177 excited by BAY × 3702 administration whereas the rest
178 ($n = 5$) were inhibited. Baseline firing rate did not differ
179 between the two groups (3.8 ± 0.9 vs. 1.8 ± 0.7 spikes/s for
180 neurons excited and inhibited by BAY × 3702, respectively;
181 $p = 0.22$; Student's t-test). Likewise, there was no difference
182 in the range of doses that excited and inhibited pyramidal
183 neurons although excitatory effects were often observed at
184 lower doses (10–20 μg/kg i.v.). Fig. 2 show representative
185 examples of pyramidal neurons excited and inhibited by
186 BAY × 3702, respectively.
187   Of the excited neurons, BAY × 3702 had a very marked
188 effect on a subgroup of 10 neurons and a moderate effect on
189 4 other neurons. On average, excited neurons increased their
190 firing rate from 3.8 ± 0.9 (baseline) to 6.0 ± 1.1 (10 μg/kg
191 i.v.), 7.3 ± 1.3 (20 μg/kg i.v.) and 6.0 ± 1.1 spikes/s (40–80
192 μg/kg i.v.) ($n = 14$; $p < 0.001$, repeated measures ANOVA)
193 (Figs. 2 and 3). The effect of the maximal dose administered
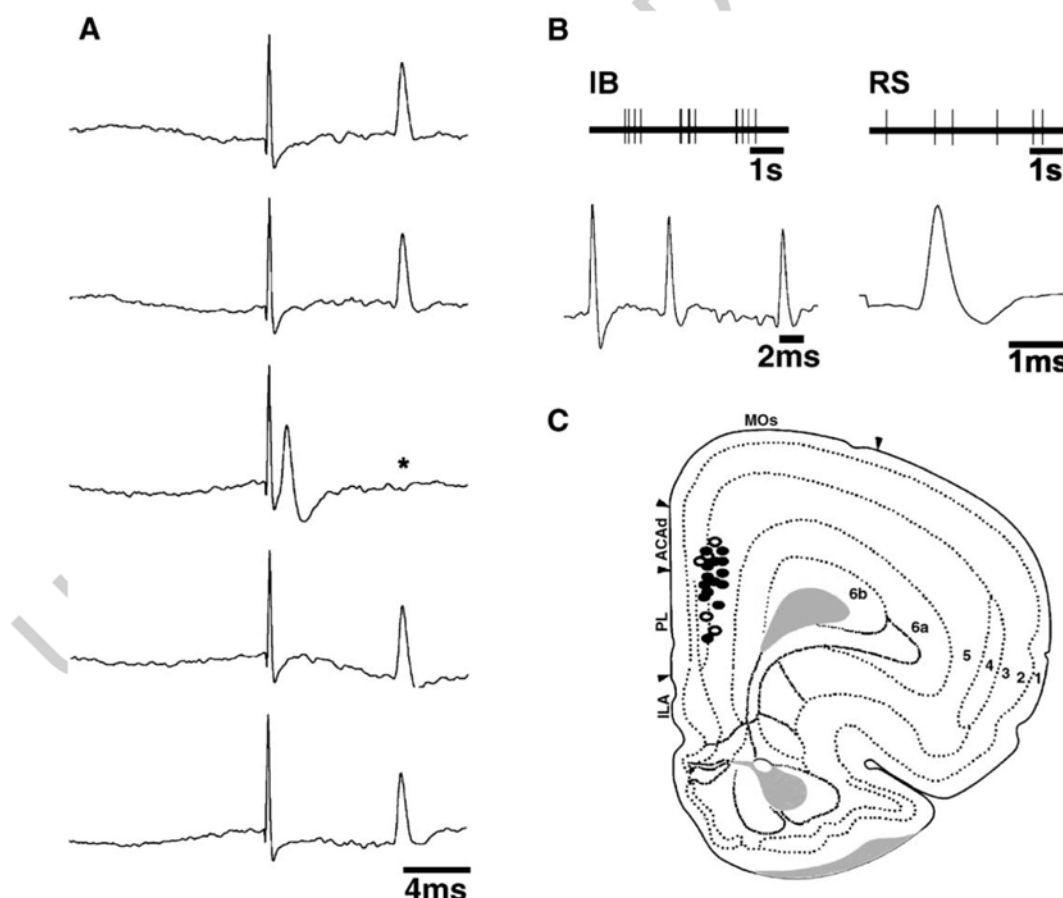194 (40–80 μg/kg i.v.) was slightly lower than that of 20 μg/kg
195 i.v.

Fig. 1. Extracellular recordings of pyramidal neuron in mPFC. A) Identification of a pyramidal projection neuron by antidromic stimulation from the ventral tegmental area (VTA). The asterisk denotes an antidromic spike missing due to collision with ongoing spontaneous action potentials. B) Representative spikes and firing patterns of projection neurons in mPFC exhibiting regular mode of firing (regular spiking, RS) or burst firing (inactivating burst firing, IB) (Dégenètais et al., 2002). C) Section drawing taken from Swanson (1998) showing the localization of the neurons recorded in the cingulate and prelimbic areas of a frontal section of the rat brain at +3.2 mm from bregma. Black and open dots show, respectively, the location of neurons excited and inhibited by BAY × 3702 administration.
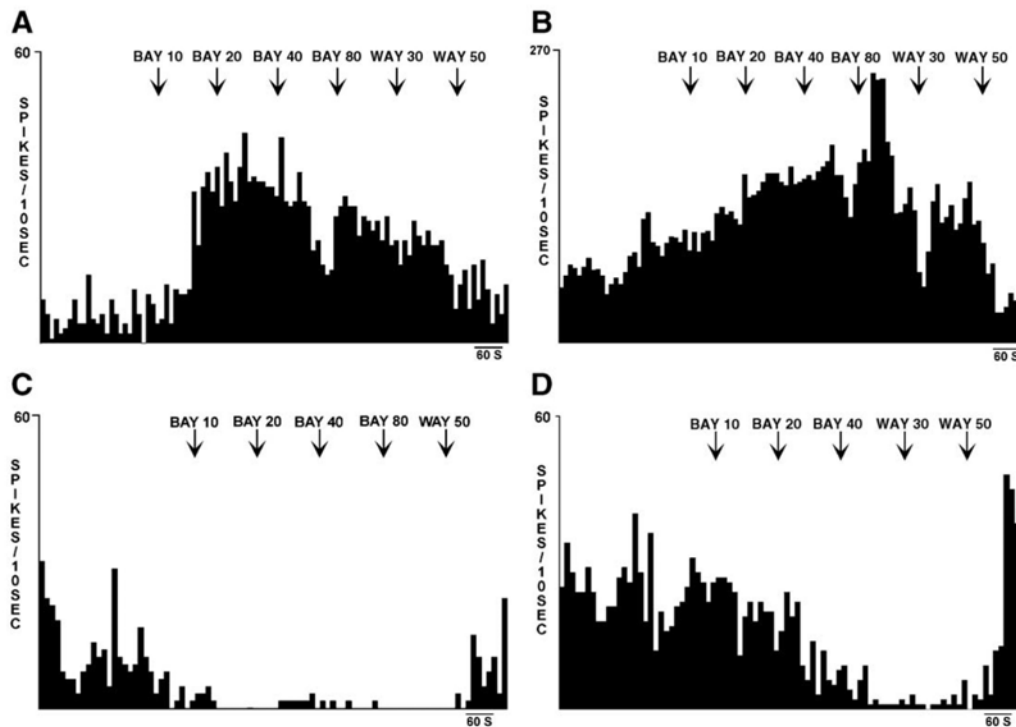
Fig. 2. Integrated firing rate histograms showing the effect of the intravenous administration of BAY × 3702 on mPFC pyramidal neurons projecting to VTA. (A, B) These neurons responded to the administration of cumulative doses of BAY × 3702 (10–80 μg/kg i.v.) with an increase in firing rate. The effect of BAY × 3702 was antagonized by the administration of the 5-HT$_{1A}$ receptor antagonist WAY-100635 (30–50 μg/kg i.v.). (C, D) Representative examples of inhibitory effects of the same dose of BAY × 3702 on pyramidal neurons of the mPFC. The two units shown in C–D had their firing rate totally suppressed by the administration of BAY × 3702 (10–80 μg/kg). The administration of WAY-100635 (30–50 μg/kg i.v.) completely reversed the inhibitory effect of BAY × 3702. Arrows mark the time of drug administration (cumulative doses).
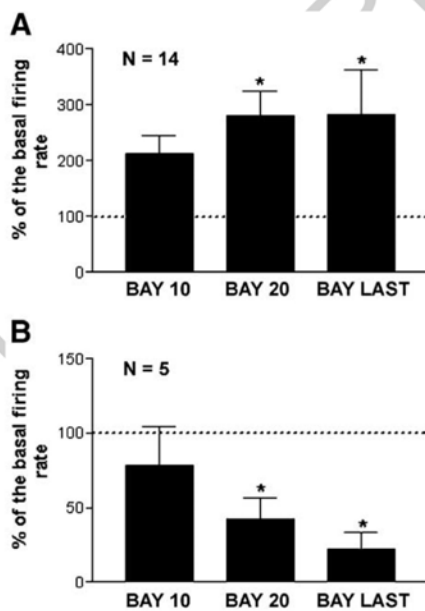

Fig. 3. Summary of the effects of BAY × 3702 on the firing rate of mPFC pyramidal neurons projecting to VTA. BAY × 3702 excited a subgroup of mPFC neurons ($n = 14$; panel A) and inhibited the rest ($n = 5$; panel B). *$p < 0.05$ versus baseline; t-test post-ANOVA. BAY LAST denotes the last dose administered to each rat; either 40 or 80 μg/kg, depending on the magnitude of the effect attained.

Similarly, at the same doses, BAY × 3702 reduced the firing rate of 5 pyramidal neurons, from 1.8 ± 0.7 (baseline) to 1.6 ± 0.9 (10 μg/kg i.v.), 1.1 ± 0.8 (20 μg/kg i.v.) and 0.7 ± 0.5 spikes/s (40–80 μg/kg i.v.) ($n = 5$; $p < 0.04$ repeated measures ANOVA) (Figs. 2 and 3). Considering all neurons ($n = 19$), BAY × 3702 increased the firing rate of pyramidal neurons to $218 \pm 41\%$ of baseline (at 20 μg/kg i.v.).

The suppressant effect of BAY × 3702 was reversed by the subsequent administration of the 5-HT$_{1A}$ antagonist WAY-100635 (30–50 μg/kg i.v.) in all neurons where reversal was attempted (from $22 \pm 11\%$ to $159 \pm 35\%$ of baseline; $n = 5$; $p < 0.03$; paired Student's t-test) (Fig. 2). We attempted to reverse the excitatory effect of BAY × 3702 in 7 neurons which were markedly activated by BAY × 3702. In 4 of them, WAY-100635 administration (up to 80 μg/kg i.v.) completely reversed the increase in firing produced by BAY × 3702 (from $288 \pm 61\%$ to $104 \pm 30\%$ of baseline, $p < 0.01$).

4. Discussion

The present study shows that the selective 5-HT$_{1A}$ receptor agonist BAY × 3702 exerts two opposite effects on the firing rate of pyramidal neurons in the mPFC

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218 projecting to VTA. All neurons examined were affected by
219 systemic BAY × 3702 administration: nearly 75% were
220 excited whereas the rest were inhibited. Since all recorded
221 neurons were identified by antidromic activation from the
222 VTA, the present results suggest that BAY × 3702 may
223 modulate the activity of DA neurons in the VTA through a
224 change in the pyramidal output to this midbrain structure.
225 This is in accordance with recent observations indicating
226 that BAY × 3702 increased the firing rate and burst firing of
227 DA neurons in the VTA, and this effect was abolished by
228 cortical transection (Díaz-Mataix et al., submitted for
229 publication).
230   Both effects of BAY × 3702 were sensitive to the
231 administration of the selective 5-HT$_{1A}$ receptor antagonist
232 WAY-100635. This, together with the very high selectivity
233 of this agent for 5-HT$_{1A}$ receptors, supports the exclusive
234 involvement of 5-HT$_{1A}$ receptors in the observed effects.
235 Indeed, the in vitro affinity of BAY × 3702 for 5-HT$_{1A}$
236 receptors is more than one and two orders of magnitude
237 higher than for α-adrenoceptors and DA D2 receptors,
238 respectively (De Vry et al., 1998). The inhibitory effect of
239 BAY × 3702 was completely reversed by WAY-100635 in
240 all neurons tested ($n=5$) whereas the excitatory effect was
241 antagonized in 4 out of 7 neurons. Although the maximal
242 dose of WAY-100635 used to antagonize both effects was
243 similar (up to 80 μg/kg i.v.), we cannot exclude the
244 possibility that higher doses were required to reverse the
245 excitatory effect of BAY × 3702, in as much as it may
246 involve an action at receptors different from those respon-
247 sible for inhibitory effects (see below). Likewise, it may be
248 that BAY × 3702 triggers a long-lasting effect on pyramidal
249 neurons beyond the initial activation of 5-HT$_{1A}$ receptors.
250   5-HT$_{1A}$ receptors are located on 5-HT neurons in the
251 midbrain raphe nuclei, where they function as autoreceptors
252 (Sprouse and Aghajanian, 1986, 1987; Blier and de
253 Montigny, 1987) and in cortical and limbic areas (Pom-
254 peiano et al., 1992). In particular, the mPFC contains a high
255 density of cells expressing 5-HT$_{1A}$ receptors (Pompeiano et
256 al., 1992; Amargós-Bosch et al., 2004). Recent studies show
257 that a large percentage ($\sim$50–60%) of pyramidal neurons
258 (as labelled by the vGLUT1 mRNA) and $\sim$20% of
259 GABAergic interneurons (as labelled by GAD mRNA) in
260 mPFC express 5-HT$_{1A}$ receptors, in the area where the
261 present recordings were made (Santana et al., 2004). Earlier
262 electrophysiological studies have shown that the micro-
263 iontophoretic application of 5-HT or 5-HT$_{1A}$ agonists
264 suppressed the firing activity of serotonergic as well as
265 cortical and hippocampal pyramidal neurons (Sprouse and
266 Aghajanian, 1986, 1987, 1988; Blier and de Montigny,
267 1987; Ashby et al., 1994). Likewise, the stimulation of the
268 dorsal and median raphe nuclei at physiological rates
269 inhibited pyramidal neurons in mPFC, through the activa-
270 tion of 5-HT$_{1A}$ receptors (Hajós et al., 2003; Amargós-
271 Bosch et al., 2004; Puig et al., 2005). Moreover, the local
272 activation of 5-HT$_{1A}$ receptors in mPFC by BAY × 3702 or
273 the prototypical 5-HT$_{1A}$ agonist 8-OH-DPAT reduced local

274 5-HT release (Casanovas et al., 1999; Amargós-Bosch et al.,
275 2004) by an effect presumably resulting from inhibition of
276 cortical excitatory inputs to the dorsal raphe nucleus (Celada
277 et al., 2001). All this previous evidence is consistent with
278 the well-known inhibitory action of 5-HT and 5-HT$_{1A}$
279 receptor agonists on cells expressing 5-HT$_{1A}$ receptors
280 coupled to GiRK channels (Andrade et al., 1986; Williams
281 et al., 1988; Araneda and Andrade, 1991; Van den Hooff
282 and Galvan, 1992; Corradetti et al., 1996; Ehrengruber et
283 al., 1997; Schmitz et al., 1998). However, the systemic
284 administration of 8-OH-DPAT moderately *increased* the
285 firing of prefrontal cells (Borsini et al., 1995; Hajós et al.,
286 1999) and induced *c-fos* expression in mPFC (Hajós et al.,
287 1999).
288   The suppressant action of BAY × 3702 on pyramidal cell
289 firing observed herein is consistent with these previous
290 observations and most likely reflects the direct activation of
291 5-HT$_{1A}$ receptors in the recorded neurons, i.e. inhibited
292 neurons would be those expressing 5-HT$_{1A}$ receptors.
293 However, the proportion of inhibited neurons is lower than
294 that of neurons expressing 5-HT$_{1A}$ receptor mRNA in
295 mPFC (Amargós-Bosch et al., 2004; Santana et al., 2004).
296 This difference may perhaps be due to the fact that recorded
297 neurons projected to the VTA and may not be representative
298 of the general population in PFC.
299   5-HT$_{1A}$ receptors may be located in the axon hillock of
300 cortical pyramidal neurons, a crucial compartment for the
301 generation of nerve impulses (De Felipe et al., 2001; Czyrak
302 et al., 2003; Cruz et al., 2004). However, as previously
303 observed for 8-OH-DPAT on putative pyramidal neurons
304 (Borsini et al., 1995; Hajós et al., 1999), BAY × 3702
305 predominantly excited mPFC pyramidal neurons projecting
306 to VTA, an effect which cannot be attributable to the
307 activation of 5-HT$_{1A}$ receptors on the recorded neurons.
308 Both effects occurred within the same dose range and were
309 sensitive to WAY-100635, as previously observed for 8-OH-
310 DPAT (Hajós et al., 1999).
311   Given the inhibitory nature of 5-HT$_{1A}$ receptors, the
312 BAY × 3702-induced excitations may actually be disinhibi-
313 tions mediated by 5-HT$_{1A}$ receptors in other cells or brain
314 areas controlling the activity of the recorded neurons. The
315 widespread distribution of 5-HT$_{1A}$ receptors in rat brain
316 opens several possibilities. A first possibility is that the
317 excitatory effect resulted from the suppression of seroto-
318 nergic activity produced by BAY × 3702 (Dong et al., 1998;
319 Casanovas et al., 2000) and a subsequent drop of the 5-HT
320 tone on prefrontal inhibitory receptors controlling pyramidal
321 activity (e.g. 5-HT$_{2A}$ and 5-HT$_3$ receptors located on
322 GABAergic interneurons; Tanaka and North, 1993; Zhou
323 and Hablitz, 1999; Puig et al., 2004; Santana et al., 2004).
324 However, the ED50 for the suppression of serotonergic cell
325 firing in the dorsal raphe nucleus is 0.5–1 μg/kg i.v. and full
326 suppression of firing occurred at 2–4 μg/kg i.v. (Dong et al.,
327 1998; Casanovas et al., 2000). This dose is below the
328 threshold to modulate pyramidal cell activity (>10 μg/kg
329 i.v.) which suggests the additional involvement of postsyn-

330 aptic 5-HT$_{1A}$ receptors, which exhibit a lower sensitivity to
331 agonists (Sprouse and Aghajanian, 1988). In parallel with
332 the present results, the dose of 8-OH-DPAT suppressing the
333 activity of 5-HT neurons is more than one order of
334 magnitude lower than that activating VTA DA neurons
335 (Lejeune et al., 1997). However, the putative involvement of
336 presynaptic 5-HT$_{1A}$ autoreceptors in the activation of
337 pyramidal neurons should be assessed in more detail using
338 5-HT-depleted animals, as examined in VTA DA neurons
339 (Prisco et al., 1994).

340 The excitatory effect of BAY × 3702 might also result
341 from the activation of 5-HT$_{1A}$ receptors in mPFC GABAer-
342 gic neurons (Santana et al., 2004), resulting in the
343 suppression of local inhibitory inputs (Fig. 4). The putative
344 involvement of a GABA$_A$ receptor-mediated disinhibitions
345 is consistent with the more marked effects of GABA$_A$
346 receptors (increasing g_{Cl}) compared with 5-HT$_{1A}$ receptors
347 (increasing g_K) on neuronal activity. Hence, GABA$_A$-
348 mediated disinhibitions may overcome the inhibitory effect
349 produced by the direct activation of 5-HT$_{1A}$ receptors on the
350 recorded neurons.

351 Likewise, BAY × 3702 may modulate the activity of PFC
352 pyramidal neurons distally, through the activation of 5-
353 HT$_{1A}$ receptors in other brain areas anatomically or
354 functionally related to the PFC (Groenewegen and Uylings,
355 2000). Among these, the hippocampal formation may play
356 an important role since i) the entire hippocampal formation
357 and afferent areas such as the entorhinal cortex and septum
358 are very rich in 5-HT$_{1A}$ receptors (Pompeiano et al., 1992),

359 ii) the CA1 subfield and the subiculum project via a direct
360 pathway to the mPFC, including the prelimbic area where
361 present recordings were made (Swanson, 1981; Jay et al.,
362 1989; Gabbott et al., 2002), and iii) pyramidal neurons from
363 CA1 and subiculum control the activity of pyramidal
364 neurons in mPFC (Thierry et al., 2000; Tierney et al.,
365 2004) either directly or through GABAergic interneurons
366 (Gabbott et al., 2002; Tierney et al., 2004). Interestingly,
367 unlike those in mPFC, hippocampal pyramidal cells respond
368 invariably with inhibitions to both the local and systemic
369 administration of 5-HT$_{1A}$ receptor agonists (Sprouse and
370 Aghajanian, 1988; Romero et al., 1996; Tada et al., 1999).
371 The putative circuitry involved in these effects is schemat-
372 ically shown in Fig. 4. The excitatory (or disinhibitory)
373 effect of BAY × 3702 on mPFC pyramidal neurons might
374 therefore be produced by an inhibitory effect on CA1/
375 subiculum neurons projecting to GABAergic interneurons
376 in mPFC. In support of this assumption, Tierney et al.
377 (2004) reported that 70% of identified GABAergic neurons
378 in mPFC responded with single action potentials or bursts to
379 single pulse stimulation of CA1/subiculum. When pairs of
380 neurons (pyramidal and GABAergic) were occasionally
381 recorded, a rapid, monosynaptic excitation of the GABAer-
382 gic neuron together with a prolonged inhibition of the
383 pyramidal neuron were observed (Tierney et al., 2004).

384 In common with other selective 5-HT$_{1A}$ receptor agonists
385 (Arborelius et al., 1993; Lejeune et al., 1997; Lejeune and
386 Millan, 1998, 2000; Sakaue et al., 2000), the systemic
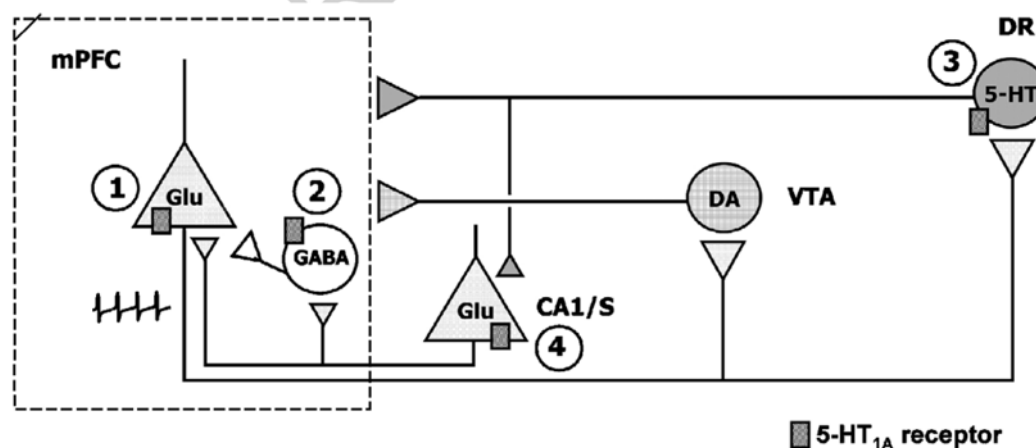387 administration of BAY × 3702 (10–40 µg/kg i.v.) increased

Fig. 4. Schematic representation of putative sites of action of BAY × 3702. (1) Inhibitory effects are likely mediated by a direct action on pyramidal neurons expressing 5-HT$_{1A}$ receptors. (2–4) Excitatory effects may possibly be accounted for by an action at various sites in brain. On the one hand, activation of 5-HT$_{1A}$ receptors on GABA interneurons in mPFC (Santana et al., 2004) may disinhibit pyramidal neurons from local GABA inputs (2). Likewise, activation of 5-HT$_{1A}$ receptors in serotonergic cell bodies reduces serotonergic tone on postsynaptic receptors putatively involved in the control of pyramidal neurons in mPFC (3). The latter may include terminal 5-HT$_{1B}$ heteroceptors as well as 5-HT$_{2A}$ and 5-HT$_3$ receptors on GABAergic neurons, whose activation by 5-HT inhibits pyramidal cell activity (Tanaka and North, 1993; Zhou and Hablitz, 1999). Finally, BAY × 3702 may increase pyramidal activity by an action at postsynaptic receptors located in distal areas projecting to mPFC, such as the hippocampal formation and afferent areas (4). 5-HT$_{1A}$ agonists have been shown to suppress the activity of CA1 pyramidal neurons after systemic administration (Tada et al., 1999). Since the CA1/subiculum sends excitatory afferents to PFC, a similar action would be expected in cortical pyramidal neurons. However, a recent study reported that 70% of the GABAergic interneurons in the mPFC were excited monsynaptically by CA1/subiculum. This raises the possibility that pyramidal neurons in mPFC can be disinhibited after the suppression of firing of CA1/subiculum neurons produced by the 5-HT$_{1A}$ agonist.

DA cell firing and DA release in mPFC (Díaz-Mataix et al., submitted for publication). Here we show that BAY × 3702 induced an overall increase of the activity of pyramidal neurons in mPFC projecting to VTA at the same doses. Although no definite causal evidence can be drawn from these results, this parallelism suggests that the change in DA cell activity is driven by the observed effects in mPFC projection neurons.

In summary, we show that the exogenous activation of 5-HT$_{1A}$ receptors by BAY × 3702 exerts a biphasic effect on the activity of prefrontal pyramidal neurons projecting to VTA, with a marked overall increase in activity. Given the similarity of actions of 5-HT$_{1A}$ agonists and atypical antipsychotics on the mesocortical DA system, the present results may help to elucidate the neurobiological mechanisms involved in the elevation of DA release produced by the latter agents.

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