Psychopharmacology (2007) 194:11–20
DOI 10.1007/s00213-007-0810-3

ORIGINAL INVESTIGATION

Paradoxical constitutive behavioral sensitization to amphetamine in mice lacking 5-HT$_{2A}$ receptors

Lucas Salomon · Christophe Lanteri · Gérard Godeheu · Gérard Blanc · Jay Gingrich · Jean-Pol Tassin

Received: 10 January 2007 / Accepted: 19 April 2007 / Published online: 19 May 2007

Abstract
Rationale Although locomotor response to *d*-amphetamine is considered as mediated by an increased release of dopamine in the ventral striatum, blockade of either α1b-adrenergic or 5-HT$_{2A}$ receptors almost completely inhibits *d*-amphetamine-induced locomotor response in mice. In agreement with this finding, mice lacking α1b-adrenergic receptors hardly respond to *d*-amphetamine. However, we show here that, paradoxically, mice lacking 5-HT$_{2A}$ receptors (5-HT$_{2A}$-R KO) exhibit a twofold higher locomotor response to *d*-amphetamine than wild-type (WT) littermates.
Objectives To explore why there is a discrepancy between pharmacological and genetic 5-HT$_{2A}$ receptor blockade.
Materials and methods Locomotor response and behavioral sensitization to *d*-amphetamine were measured in presence of prazosin and/or SR46349B, α1b-adrenergic, and 5-HT$_{2A}$ receptor antagonists, respectively.
Results Repeating amphetamine injections still increases 5-HT$_{2A}$-R KO mice locomotor response to *d*-amphetamine at a level similar to that of sensitized WT mice. SR46349B (1 mg/kg) has, as expected, no effect in 5-HT$_{2A}$-R KO mice. One milligrams per kilogram of prazosin completely blocks *d*-amphetamine-induced locomotor response in 5-HT$_{2A}$-R KO naïve animals but 3 mg/kg is necessary in sensitized 5-HT$_{2A}$-R KO mice.
Conclusions Because naïve 5-HT$_{2A}$-R KO mice exhibit an increased cortical noradrenergic response to *d*-amphetamine, our data suggest that repeated *d*-amphetamine modifies noradrenergic transmission in 5-HT$_{2A}$-R KO mice. Stimulation of specific 5-HT$_{2A}$ receptors would inhibit noradrenergic neurons. Dramatic decrease in SR46349B efficiency in sensitized WT mice indicates that a disruption of the regulating role of 5-HT$_{2A}$ receptors on noradrenergic transmission occurs during sensitization and thus represents the physiological basis of behavioral sensitization to *d*-amphetamine.

Keywords 5-HT$_{2A}$-Serotonergic receptor · α1b-Adrenergic receptor · *d*-Amphetamine · Locomotor activity · Behavioral sensitization · Knock-out mice

L. Salomon · C. Lanteri · G. Godeheu · G. Blanc · J. Gingrich · J.-P. Tassin (✉)
CNRS UMR 7148, Collège de France,
11, Place Marcelin Berthelot,
75231 Paris Cedex 05, France
e-mail: jean-pol.tassin@college-de-france.fr

J. Gingrich
Columbia University New York State Psychiatric Institute,
Dev Psychobiol, Neurobiol, & Behavior,
722 West 168th Box 40,
New York, NY 10032, USA

Introduction

Behavioral consequences of *d*-amphetamine injections are generally considered as the consequence of an increased release of dopamine (DA) in the brain. This increased DA release is due to the blockade or the reversal of the DA transporter located on dopaminergic nerve terminals (Besson et al. 1971; Von Voigtlander and Moore 1973). Because the locomotor hyperactivity induced by the systemic injection of *d*-amphetamine is inhibited either by bilateral 6-hydroxydopamine lesions of mesolimbic dopaminergic neurons or by the application of neuroleptics into the nucleus accumbens, it was suggested that *d*-amphetamine-induced psychomotor activation mainly results from an increased DA transmission in this structure (Pijnenburg et al. 1975; Kelly et al. 1975). However, *d*-amphetamine is

also a potent norepinephrine (NE) releaser and many studies have indicated that blocking noradrenergic transmission with an α1-adrenergic antagonist, such as prazosin, hampers *d*-amphetamine-induced locomotor hyperactivity (Snoddy and Tessel 1985; Dickinson et al. 1988; Blanc et al. 1994). More recently, it was shown that mice lacking the α1b-adrenergic receptor (α1b-AR KO) were severely affected in their locomotor response and behavioral sensitization to *d*-amphetamine (Drouin et al. 2002). Finally, microdialysis experiments indicated that the increased release of DA induced by *d*-amphetamine in the nucleus accumbens was absent in α1b-AR KO mice (Auclair et al. 2002). Altogether, these data confirmed the hypothesis of the existence of interactions between noradrenergic and dopaminergic neurons (Antelman and Caggiula 1977; Kokkinidis and Anisman 1978, 1979; Ogren et al. 1983; Tassin et al. 1986; Taghzouti et al. 1988; Lategan et al. 1990; Shi et al. 2000; Drouin et al. 2002) and, more precisely, of a coupling between noradrenergic and dopaminergic transmissions (Darracq et al. 1998).

Further experiments nevertheless indicated that, even in α1b-AR KO mice, *d*-amphetamine was still inducing a slight locomotor response, suggesting that other factors than noradrenergic and dopaminergic transmissions were implied in *d*-amphetamine-induced behavioral effects. Because some data had shown that the systemic injection of MDL 100907, a specific 5-HT_{2A} receptor antagonist, reduces the locomotor hyperactivity induced by amphetamine (Moser et al. 1996; Sorensen et al. 1993; O'Neill et al. 1999), 5-HT_{2A} receptors appeared good candidates to participate in the behavioral effects of *d*-amphetamine. Moreover, Porras et al. (2002) and Auclair et al. (2004a), using another 5-HT_{2A} receptor antagonist, SR46349B (Rinaldi-Carmona et al. 1992), have found that the blockade of 5-HT_{2A} receptors decreases the *d*-amphetamine-induced increase in extracellular DA levels in the nucleus accumbens. Finally, in more recent experiments, we found that 5-HT_{2A} and α1b-adrenergic receptors entirely mediate not only DA release but also locomotor response and behavioral sensitization induced by *d*-amphetamine (Auclair et al. 2004b). These data therefore suggested that both receptors participate in the same regulating pathway.

To test this hypothesis, i.e., whether 5-HT_{2A} receptors control *d*-amphetamine-induced locomotor activity in a way similar to that observed for α1b-adrenergic receptors, mice deprived of 5-HT_{2A} receptors (5-HT_{2A}-R KO) were studied. Their locomotor responses after acute and repeated treatment with *d*-amphetamine were monitored and compared to those obtained in wild-type (WT; 5-HT_{2A} +/+) and α1b-AR KO mice. The effects of antagonists of α1b-adrenergic and 5-HT_{2A} receptors, prazosin and SR46349B, respectively, were also assessed in 5-HT_{2A}-R KO and WT mice. Finally, because effects of prazosin and SR46349B on *d*-amphetamine-induced locomotor hyperactivity were different between naïve and sensitized mice, dose–response curves for both receptor antagonists were performed. Data indicate that 5-HT_{2A} receptors blockade do not have the same consequences in naïve mice than in those sensitized to *d*-amphetamine.

Materials and methods

Animals

Experiments were performed on adult male mice (25–35 g). 5HT_{2A}-R KO mice (Fiorica-Howells et al. 2002; Weisstaub et al. 2006) were back-crossed on a C57BL6 genetic background for at least seven generations. The homozygous 5-HT_{2A} +/+ mice were used as controls all over the study and referred to WT mice in the text. α1b-AR KO mice (Cavalli et al. 1997) were also back-crossed on a C57Bl6 genetic background for at least seven generations. When α1b-AR KO were used (see Fig. 2), it was verified that locomotor response to amphetamine was similar in homozygous α1b-AR +/+ and 5-HT_{2A} +/+ mice. All mice, housed by groups of eight in plastic cages, were maintained on a 12-h light/dark cycle (lights on at 07:00) with food and water freely available.

Drugs

d-Amphetamine sulfate (Sigma Aldrich, L'Isle d'Abeau-Chesne, France) was dissolved in saline. Prazosin hydrochloride (Sigma Aldrich) was sonicated in water. SR46349B hemifumarate {(1Z,2E)-1-(2-fluoro-phenyl)-3-(4-hydroxyphenyl)-prop-2-en-one-O-(2-dimethylamino-ethyl)-oxime hemifumarate} was a generous gift from Laboratories Sanofi-Synthelabo (Montpellier, France). It was dissolved with a drop of lactic acid, neutralized with 1 M of NaOH, and sonicated in saline. All drugs were injected intraperitoneally (0.3 ml per 100 g). Doses are expressed as salts. *d*-Amphetamine was given at 2 mg/kg i.p. When not specifically specified, doses of prazosin (1 mg/kg, i.p.) and SR46349B (1 mg/kg, i.p.) were kept identical to previous experiments (Auclair et al. 2004a, b; Drouin et al. 2002; Salomon et al. 2006).

Locomotor activity

Acute treatment Mice were introduced in a circular corridor (4.5 cm width, 17 cm external diameter) crossed by four infrared beams (1.5 cm above the base) placed at every 90° (Imetronic, Pessac, France). The locomotor activity was counted when animals interrupted two successive beams and thus had traveled a quarter of the circular corridor. In each session, the spontaneous activity was recorded for 60 min (habituation to the experimental procedure). Then,

mice received intraperitoneal saline or pretreatment (prazosin or/and SR46349B), and their activity was recorded for 30 min. Finally, mice were injected intraperitoneally with *d*-amphetamine (2 mg/kg), and their locomotor responses were recorded for an additional 120-min period. Doses of *d*-amphetamine were chosen as the highest ones, which never induced stereotypy after repeated treatments.

Repeated treatment Mice were injected on four consecutive days, and their locomotor activity was recorded with the same protocol as that for an acute treatment. To test the effect of the pretreatment on the *expression* of behavioral sensitization, mice received every single day only saline and *d*-amphetamine (2 mg/kg). Four days after the last injection, mice received prazosin (1 mg/kg) and/or SR46349B (1 mg/kg) followed 30 min later by *d*-amphetamine. To test the effect of the pretreatment on the *development* of behavioral sensitization, mice received every single day a pretreatment (prazosin [1 mg/kg] and/or SR46349B [1 mg/kg]) plus *d*-amphetamine. Four days after the last injection, mice received only saline and a *d*-amphetamine injection.

Statistics

Statistical analysis was performed using Graph Pad Prism 3.0 software, (San Diego, CA). Locomotor activity was described either in function of time or in function of pretreatment. Two-way ANOVA (genotype×treatment) were performed to study the effects of the different parameters and their possible interactions on the whole acute and repeated responses on 1 h of the three mice strains. Bonferroni posttests were done to compare more precisely the different strains. To study the global effect of treatments and pretreatments in naïve and sensitized animals and during the development of behavioral sensitization, means of the locomotor activity were as well calculated per 1 h and compared with one-way ANOVA followed by Tukey's test. Pharmacological treatments correspond to independent groups of animals. Significant differences were set at $P<0.05$.

Results

$5HT_{2A}$-R KO mice exhibit higher locomotor response to *d*-amphetamine than WT mice; effects of prazosin and SR46349B

d-Amphetamine (2 mg/kg) induced increased locomotion in both WT and $5HT_{2A}$-R KO mice [two-way ANOVA (genotype×treatment) $F(2,39)=15.66$; $P<0.0001$]. Interestingly, we found also a significant effect of genotype ($F(1,39)=14.64$; $P<0.0005$) and the interaction between genotype and treatment ($F(2,39)=4.46$; $P=0.0118$). More precisely, $5HT_{2A}$-R KO mice were surprisingly found hyperreactive to *d*-amphetamine when compared with WT littermates (Bonferroni posttest: $t=2.83$; $P<0.05$). This difference was not related to the hyperreactivity of $5HT_{2A}$-R KO mice to novelty when compared with WT mice (Student's *t* test $t=0.71$; $P=0.5$) nor to an effect of stress induced by injection (Fig. 1). As expected, in WT mice, pretreatment changed the locomotor response to *d*-amphetamine (one-way ANOVA $F(2,21)=9.723$; $P=0.001$). Prazosin (1 mg/kg), a α1-adrenergic receptor antagonist, decreased WT mice locomotor response to *d*-amphetamine by 93% (Tukey's posttest; $P<0.01$). SR46349B (1 mg/kg), a selective $5HT_{2A}$ receptor antagonist, induced an 80% loss of the locomotor response (Tukey's posttest; $P<0.01$). In $5HT_{2A}$-R KO mice, pretreatment also modified the locomotor response (one-way ANOVA $F(2,18)=9.696$; $P=0.0014$). Prazosin completely blocked the important locomotor effects of *d*-amphetamine (−99%, Tukey's posttest; $P<0.01$); however, SR46349B did not modify, as expected, the locomotor response induced by *d*-amphetamine (Tukey's posttest; $P>0.05$), strongly suggesting that, in our conditions, SR46349B is specifically acting on 5-HT_{2A} receptors.

Similar behavioral sensitization to *d*-amphetamine in WT and $5HT_{2A}$-R KO mice but not in α1b-AR KO mice

Figure 2 presents the time courses of behavioral sensitization to *d*-amphetamine in three strains of mice. As shown on Fig. 1, *d*-amphetamine (2 mg/kg) induced an increased locomotion, which increased with repeated injections [two-way ANOVA (genotype×treatment) $F(4,90)=8.60$; $P<0.0001$ for the treatment effect]. A significant effect of genotype was found ($F(2,90)=61.55$; $P<0.0001$) but no interaction between the genotype and treatment. Comparing the acute response, the three strains responded differently (one-way ANOVA, $F(2,20)=8.23$; $P=0.023$). As shown on Fig. 1, 5-HT_{2A}-R KO mice exhibited a higher locomotor response than WT mice (+110%, Tukey's posttest, $P<0.05$). On the contrary, and in agreement with previous data, *d*-amphetamine induced a lower locomotor activity in α1b-AR KO mice than in WT mice (−63%, Tukey's posttest; $P<0.05$). After four daily injections and a 4-day withdrawal period, the *d*-amphetamine test injection induced a potentiated locomotor activity in WT mice [+292%, one-way ANOVA, $F(4,35)=7.2$; $P=0.0002$] and in $5HT_{2A}$-R KO mice [+93%, one-way ANOVA $F(4,28)=2.72$; $P=0.05$] but not in α1b-AR KO mice [one-way ANOVA, $F(4,27)=2.72$; $P=0.56$], revealing a behavioral sensitization in the two first strains but not in the third one.

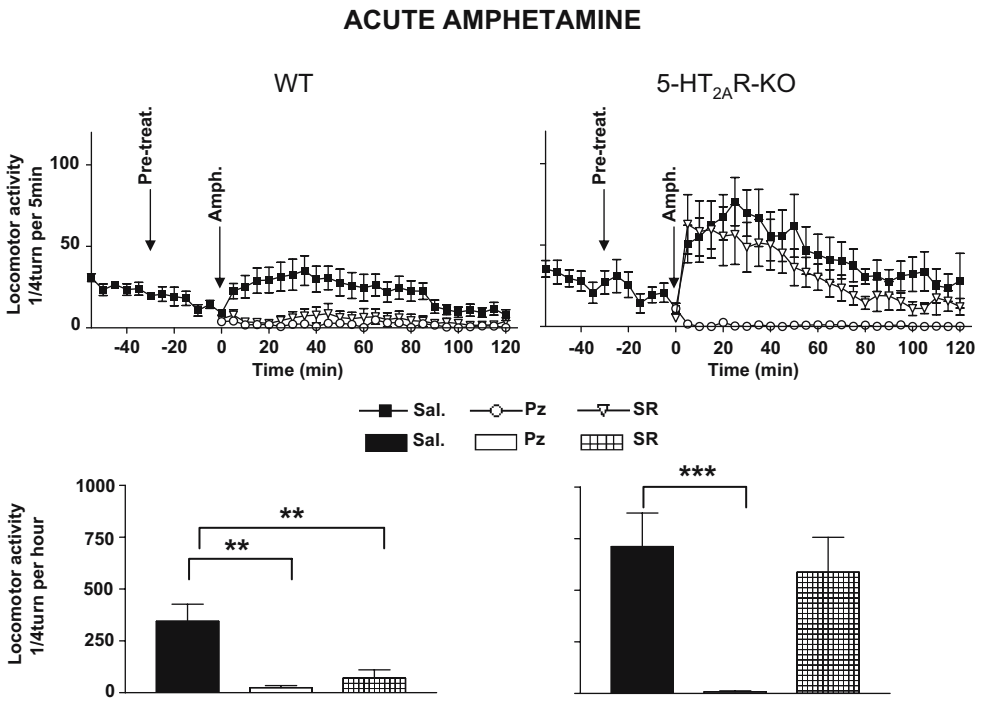

Fig. 1 Effects of prazosin and SR46349B on the acute *d*-amphetamine-induced locomotor response in WT and 5-HT_{2A}-R KO mice. Prazosin (1 mg/kg, i.p.), SR46349B (1 mg/kg, i.p.), or saline were injected 30 min before *d*-amphetamine treatment (2 mg/kg, i.p.). Locomotor activity is expressed as quarter turns per 5 min and is presented as histograms of locomotor activity during 60 min after *d*-amphetamine injections. Saline injection that induces a very small locomotor response in both strains is not shown for sake of clarity. Each group contained at least seven and no more than nine animals. *Double asterisks* indicate $P<0.01$, and *triple asterisks* indicate $P<0.001$ significantly different from corresponding controls

Interestingly, after behavioral sensitization to *d*-amphetamine, whereas locomotor response to *d*-amphetamine in the three strains was still different [one-way ANOVA, $F(2,20)=8.23$; $P=0.023$], locomotor response to *d*-amphetamine was similar in $5HT_{2A}$-R KO and WT mice (Tukey's posttest; $P>0.05$). Locomotor response to *d*-amphetamine in sensitized α1b-AR KO mice was found to be significantly lower than in sensitized WT mice (−80%, Tukey's posttest; $P<0.001$).

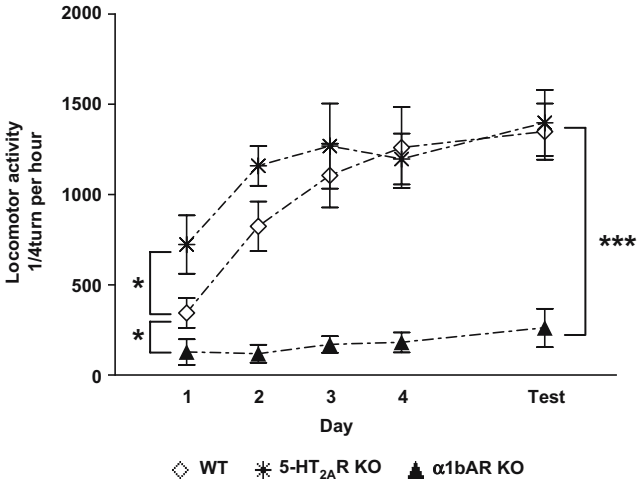

Fig. 2 Development of behavioral sensitization to *d*-amphetamine in WT, 5-HT_{2A}-R KO, and α1b-AR KO mice. Mice were given every day one injection of *d*-amphetamine (2 mg/kg) and received, after a 4-day withdrawal, a last injection of *d*-amphetamine (2 mg/kg) on the test day. Locomotor activity is expressed as the sum of quarter turns per 60 min after *d*-amphetamine injection. Each group contained at least seven and no more than nine animals. An *asterisk* indicates $P<0.05$, and *triple asterisks* indicate $P<0.001$ significantly different from corresponding controls

Effects of prazosin and SR46349B in sensitized $5HT_{2A}$-R KO and WT mice

Repeating amphetamine injections induces an increase in locomotor response to *d*-amphetamine in both WT and 5-HT_{2A}-R KO mice (Fig. 3). In sensitized mice, significant effects of the treatment and the genotype [two-way ANOVA (genotype×treatment), treatment: $F(2,37)=22.50$; $P<0.0001$ and genotype: $F(1,37)=5.01$; $P=0.03$] were found, but no interaction was revealed between the two parameters. As described above, after repeated treatment with *d*-amphetamine, $5HT_{2A}$-R KO and WT mice exhibited the same locomotor response to *d*-amphetamine (Bonferroni posttest: $t=0.24$; $P>0.05$). In WT mice, pretreatments modified significantly the locomotor responses to *d*-amphetamine [one-way ANOVA, $F(2,21)=10.21$; $P=0.0008$]. Prazosin (1 mg/kg) and SR46349B (1 mg/kg) partially blocked the locomotor response to *d*-amphetamine (−67%, Tukey's posttest; $P<0.001$ and −39%; $P<0.05$, for prazosin and SR46349B, respectively). We also found a

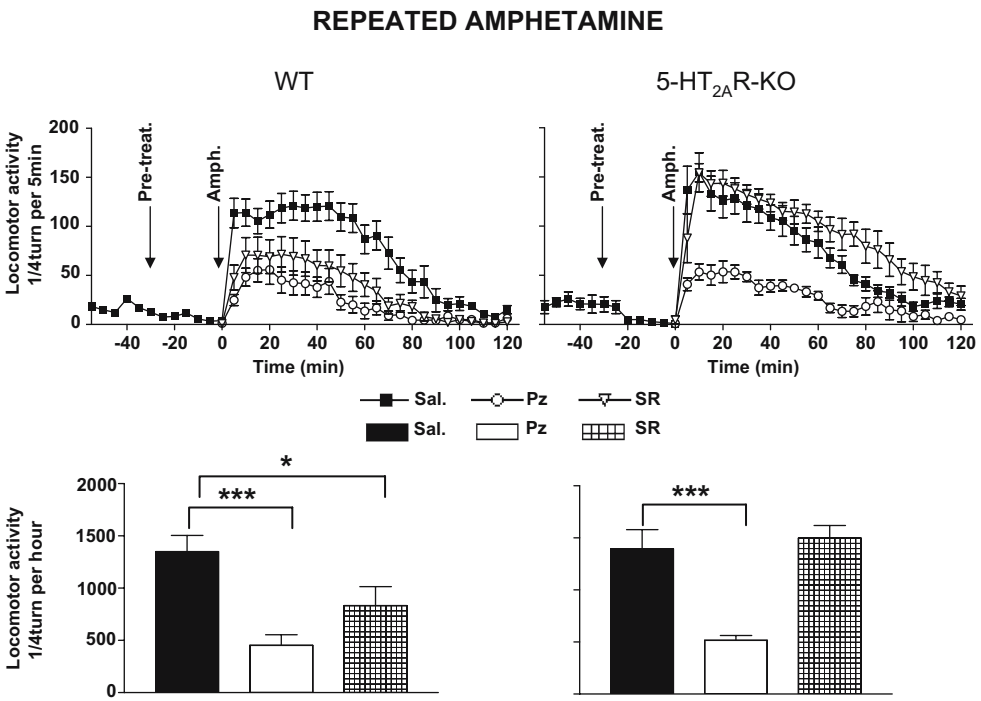

Fig. 3 Effects of prazosin and SR46349B on the repeated *d*-amphetamine-induced locomotor activity in WT and 5-HT_{2A}-R KO mice. Prazosin (1 mg/kg, i.p.), SR46349B (1 mg/kg, i.p.), or saline were injected 30 min before *d*-amphetamine treatment (2 mg/kg, i.p.) on the test day. Locomotor activity is expressed in quarter turns per 5 min and is presented as histograms of locomotor activity during 60 min after *d*-amphetamine injections. Each group contained at least seven and no more than nine animals. An *asterisk* indicates $P<0.05$, and *triple asterisks* indicate $P<0.001$ significantly different from corresponding controls

significant effect of pretreatment in 5-HT_{2A}-R KO mice [one-way ANOVA, $F(2,17)=17.37$; $P<0.0001$]. Interestingly, SR46349B had no effect (Tukey's posttest $P>0.05$); however, prazosin, in contrast to that obtained in naïve animals (Fig. 1), only reduced the locomotor response by 63% (Tukey's posttest; $P<0.001$).

Dose–response curves of the effects of prazosin and SR46349B on locomotor responses to *d*-amphetamine in naïve and sensitized 5-HT_{2A}-R KO and WT mice

To analyze why prazosin does not completely block locomotor response to *d*-amphetamine in sensitized 5HT_{2A}-R KO mice, dose–response curves were performed in naïve and sensitized 5HT_{2A}-R KO and WT mice. Figure 4 summarizes data obtained after injection of prazosin or SR46349B in WT and 5-HT_{2A}-R KO mice. It confirms that each antagonist, prazosin or SR46349B, blocks by more than 80% *d*-amphetamine-induced locomotor response in naïve WT mice (80+80%>100%, which makes the sum of both effects more than additive), whereas these effects are smaller and become additive (respectively, 63 and 39%, which makes almost 100%) in sensitized WT mice. Increasing the dose of prazosin from 1 to 3 mg/kg does not lead to a higher inhibition of locomotor response to *d*-amphetamine in sensitized and naïve WT mice (Tukey's posttest; $P>0.05$ in both cases). Finally, it is confirmed on this figure that, as shown on Figs. 1 and 3 and up to 1 mg/kg, SR46349B has no effect on *d*-amphetamine-induced locomotor response in 5-HT_{2A}-R KO mice. In 5-HT_{2A}-R KO naïve mice, 1 mg/kg of prazosin blocks completely *d*-amphetamine-induced locomotor response, an effect which is similar to that obtained with 3 mg/kg of prazosin [one-way ANOVA, $F(3,24)=21.16$; $P<0.0001$; Tukey's posttest between 1 and 3 mg/kg of prazosin; $P>0.05$]. In 5-HT_{2A}-R KO sensitized mice, a complete inhibition of *d*-amphetamine-induced locomotor response is only obtained with 3 mg/kg of prazosin, an effect significantly different from the partial inhibition now obtained with 1 mg/kg of prazosin [−63%, one-way ANOVA, $F(3,24)=28.22$; $P<0.0001$; Tukey's posttest between 1 and 3 mg/kg of prazosin; $P<0.05$).

Complete blockade of the development of behavioral sensitization to *d*-amphetamine is obtained with prazosin (1 mg/kg) in 5HT_{2A}-R KO mice but necessitates the addition of SR46349B (1 mg/kg) to prazosin in WT mice

In WT mice, pretreatment with prazosin (1 mg/kg) 30 min before the *d*-amphetamine injection modifies significantly the locomotor sensitization to *d*-amphetamine [one-way ANOVA, $F(5,36)=16.68$; $P<0.0001$] and leads, on the test day, to a partial sensitization of the mice (+126%, Tukey's posttest $P<0.05$ when compared to acute *d*-amphetamine and −43%, $P<0.05$ when compared to repeated saline/*d*-amphetamine; Fig. 5a). The same protocol with the 5HT_{2A}-R KO mice was sufficient to completely block the development of the behavioral sensitization [one-way ANOVA, $F(5,36)=21.74$ $P<0.0001$; Tukey's posttest $P>0.05$ when compared to acute *d*-amphetamine). Interestingly, the WT mice pretreated with prazosin showed on the test day a response to *d*-amphetamine totally similar to that

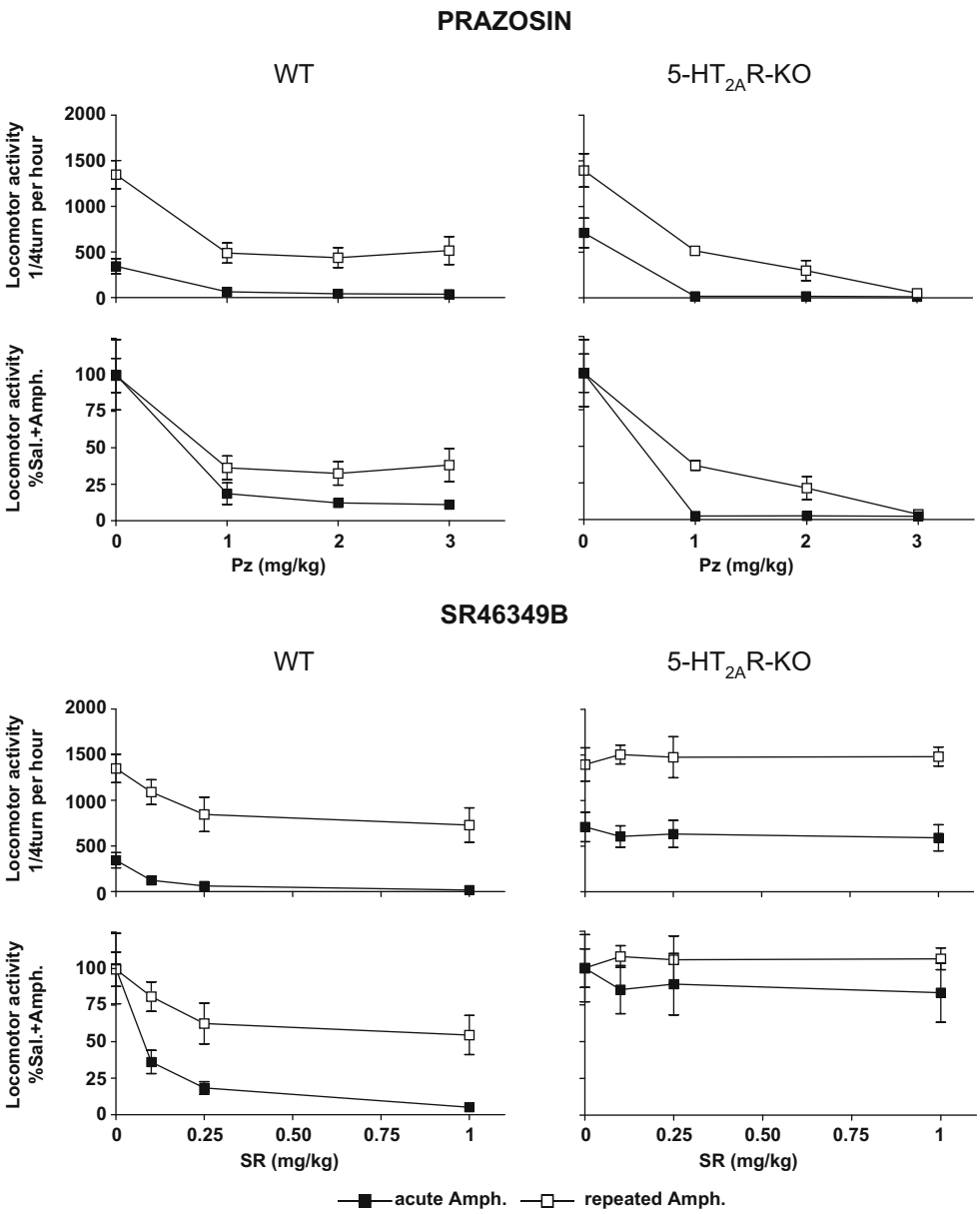

Fig. 4 Prazosin and SR46349B pretreatment dose–responses curves in *d*-amphetamine naïve and sensitized WT and 5-HT_{2A}-R KO mice. Prazosin (0–3 mg/kg, i.p.), SR46349B (0–1 mg/kg, i.p.), or saline were injected 30 min before *d*-amphetamine treatment (2 mg/kg, i.p.). This figure shows, among other points, that increasing doses of prazosin from 1 to 3 mg/kg does not modify its inhibitory effect in sensitized WT mice whereas it does in 5-HT_{2A}-R KO mice. Locomotor activity is expressed as the sum of quarter turns per 60 min after *d*-amphetamine injection. Each group contained at least seven and no more than nine animals

obtained in naïve 5HT_{2A}-R KO mice ($t_{(1, 10)}$=0.25, P=0.80, Student's t test). The addition of SR46349B (1 mg/kg) to prazosin in the pretreatment of WT mice (Fig. 5b) completely blocked the development of behavioral sensitization [one-way ANOVA, $F(5,39)$=23.36 P<0.0001; Tukey's posttest P>0.05 when compared to acute *d*-amphetamine), in agreement with previous data (Auclair et al. 2004b).

Discussion

When animals receive an injection of a α1-adrenergic receptor antagonist before a systemic administration of *d*-amphetamine, the locomotor hyperactivity usually observed after a *d*-amphetamine injection is severely affected. In agreement with this finding, α1b-AR KO mice exhibit a diminished locomotor response to *d*-amphetamine (Drouin et al. 2002). Similarly, when animals receive an injection of a 5-HT_{2A} receptor antagonist before a systemic administration of *d*-amphetamine, the *d*-amphetamine-induced locomotor response is decreased by more than 80%. We show now that, paradoxically, 5-HT_{2A}-R KO mice exhibit a twofold higher locomotor response to *d*-amphetamine than WT littermates. These data suggest that 5-HT_{2A}-R KO mice are constitutively sensitized to *d*-amphetamine.

However, despite a higher (+110%, Fig. 1) acute response to *d*-amphetamine than WT mice, *d*-amphetamine-induced locomotor activity is lower in naïve 5-

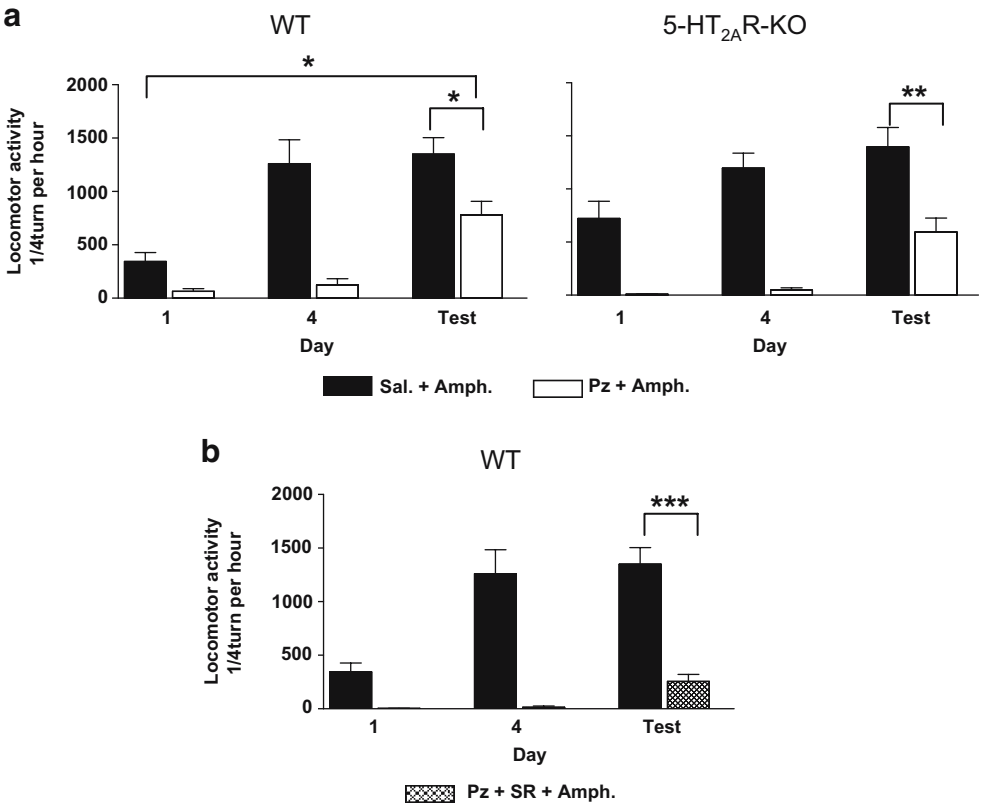

Fig. 5 Effects of prazosin alone or in combination with SR46349B on the development of behavioral sensitization to *d*-amphetamine in WT and in 5-HT_{2A}-R KO mice. In WT and 5-HT_{2A}-R KO mice, pretreatment [prazosin (1 mg/kg) or saline] was administered up to the fourth day, 30 min before *d*-amphetamine injection (**a**). In WT mice, pretreatment [prazosin+SR46349B (1 mg/kg) or saline] was administered up to the fourth day, 30 min before *d*-amphetamine injection (**b**). On the test day, only saline and *d*-amphetamine were injected. Histograms represent the sum of the locomotor activity in quarter turn per 60 min. Each group contained at least seven and no more than nine animals. An *asterisk* indicates $P<0.05$, *double asterisks* indicate $P<0.01$, and *triple asterisks* indicate $P<0.001$ significantly different from corresponding controls

HT_{2A}-R KO mice than in sensitized WT mice (−47%, Fig. 2), i.e., those having received four injections of *d*-amphetamine. Actually, repeated injections of *d*-amphetamine increase *d*-amphetamine-induced locomotor response in 5-HT_{2A}-R KO mice in such a way that these latter respond to *d*-amphetamine with the same amplitude than sensitized WT mice (Fig. 2). This means that, although 5-HT_{2A}-R KO mice appear to be constitutively sensitized, repeating *d*-amphetamine treatments can still increase their locomotor response to *d*-amphetamine, suggesting that their sensitization to *d*-amphetamine is only partial.

Analysis of dose–response curves of *d*-amphetamine-induced locomotor activity after α1-adrenergic or 5-HT_{2A} receptor antagonist treatment shows that, as expected, SR46349B, a 5-HT_{2A} receptor antagonist, has no effect on 5-HT_{2A}-R KO mice *d*-amphetamine-induced locomotor response, regardless of mice being naïve or sensitized to *d*-amphetamine. This strongly suggests that SR46349B is, at least at the doses used in this study, specific for 5-HT_{2A} receptors. Moreover, previous studies performed in rats have indicated a tenfold difference of SR46349B affinity for 5-HT_{2A} and 5-HT_{2C} receptors (Rinaldi-Carmona et al. 1992). In sensitized WT mice, it seems that *d*-amphetamine-induced locomotor response can be dissociated into two components, an α1-adrenergic one that is blocked by prazosin (−63%) and the other one being serotonergic (5-HT_{2A}) and blocked by SR46349B (−39%). In naïve WT mice, however, each antagonist blocks almost completely the *d*-amphetamine-induced locomotor response, suggesting that both locomotor components are linked or coupled. In other words, in naïve mice, blocking one locomotor component with one antagonist may block the other locomotor component, thus inhibiting completely the locomotor response. Repeated *d*-amphetamine would dissociate the link between the two locomotor components, as previously proposed (Salomon et al. 2006). This hypothesis may also explain why animals repeatedly treated with *d*-amphetamine have a higher locomotor response to *d*-amphetamine than naïve mice and thus exhibit a behavioral sensitization.

Our experiments therefore indicate that repeating *d*-amphetamine injections diminish the blocking effects of prazosin and SR46349B on *d*-amphetamine-induced locomotor response. This difference between naïve and sensitized mice response is, however, more important with regards to SR46349B than prazosin. Accordingly, when the same experiments were performed in rats, this difference between naïve and sensitized animals was only found significant with SR46349B (data not shown). This nevertheless indicates that consequences of repeated amphetamine injections on the mutual regulation between noradrenergic and serotonergic neurons are not specific to one species.

We have shown previously that naïve 5-HT_{2A}-R KO mice exhibit acutely a higher cortical NE response to *d*-amphet-

amine than WT mice and that this increased response is correlated with behavioral sensitization to *d*-amphetamine (Salomon et al. 2006). Recent experiments indicate that amphetamine sensitization does not increase further cortical NE response in 5-HT_{2A}-R KO mice (data not shown). However, 3 mg/kg of prazosin is necessary to block completely locomotor response to *d*-amphetamine in sensitized 5-HT_{2A}-R KO mice, whereas 1 mg/kg of prazosin is enough in naïve 5-HT_{2A}-R KO mice. One possibility could be that repeating amphetamine injections in 5-HT_{2A}-R KO mice has modified the affinity of α1b-adrenergic receptors for prazosin. This hypothesis is presently tested in the laboratory.

Altogether, we show that genetic deletion of 5-HT_{2A} receptors facilitates the locomotor response to *d*-amphetamine; however, this increased response can still be amplified by repeated *d*-amphetamine treatments. Behavioral sensitization to *d*-amphetamine in 5-HT_{2A}-R KO mice may therefore be partly due to a disinhibition of noradrenergic neurons related to the absence of 5-HT_{2A} receptors (constitutive sensitization) and partly to a modification of α1b-adrenergic receptors because of repeated amphetamine treatments (induced sensitization).

A role for 5-HT_{2A} receptors in the regulation of locus coeruleus noradrenergic neurons has already been described. For example, serotonin exerts a tonic inhibitory influence on locus coeruleus neurons through postsynaptic 5-HT_{2A} receptors that are not located on noradrenergic neurons (Szabo and Blier 2001). Same authors (Szabo and Blier 2002) have also shown that, whereas sustained administration of a serotonin reuptake blocker, such as citalopram or paroxetine, reduces firing activity of noradrenergic neurons, a serotonin reuptake blocker that is also a 5-HT_{2A} receptor antagonist, YM992, increases the synaptic availability of NE. Similarly, Chiang and Aston-Jones (1993) have suggested that 5-HT_{2A} receptor stimulation influences locus coeruleus indirectly and causes tonic activation of a GABAergic input to noradrenergic neurons. In agreement with this hypothesis, Szabo and Blier (2001) have proposed a localization of 5-HT_{2A} receptors on GABAergic nerve terminals in the locus coeruleus.

One question remains, however, unanswered: Despite the inhibiting effects of prazosin and SR46349B on *d*-amphetamine-induced locomotor response, why are α1b-AR KO and 5-HT_{2A}-R KO mice, respectively, hypo- and hyper-responsive to *d*-amphetamine?

The first part of the answer is that 2 mg/kg of *d*-amphetamine releases NE but has no effect on extracellular serotonin, at least in our hands (Salomon et al. 2006). Lack of α1b-adrenergic receptors annihilates the behavioral effects of *d*-amphetamine related to an increased NE release (Drouin et al. 2002); however, in 5-HT_{2A}-R KO mice, the disinhibition of noradrenergic neurons may increase the stimulation by NE of cortical α1b-adrenergic receptors (Darracq et al. 1998). The possibility that *d*-amphetamine effects are specific to noradrenergic neurons was confirmed when we tested locomotor responses to a specific serotonin releaser, *para*-chloroamphetamine (PCA). Indeed, locomotor response to PCA is more than threefold higher in α1b-AR KO mice than in WT mice (Salomon et al. 2006), thus suggesting that, in α1b-AR KO mice, serotonergic neurons are disinhibited as well as are noradrenergic neurons in 5-HT_{2A}-R KO mice.

The second part of the answer concerns the effects of SR46349B and prazosin; both compounds decrease locomotor response to *d*-amphetamine in WT mice. This means that the stimulation of α1b-adrenergic or/and 5-HT_{2A} receptors should facilitate locomotor responses to *d*-amphetamine, a finding that disagrees with data obtained in 5-HT_{2A}-R KO mice. This may indicate that the stimulation of one type of receptor has two opposite consequences depending on its localization. For example, stimulation of 5-HT_{2A} receptors increases amphetamine-induced DA release (Ichikawa and Meltzer 1995) or locomotor response through an activation of dopaminergic neurons in the ventral tegmental area (VTA; Doherty and Pickel 2000; Auclair et al. 2004a) or of pyramidal cells in the prefrontal cortex (Pazos et al. 1985; Bortolozzi et al. 2005), whereas stimulation of 5-HT_{2A} receptors located in the locus coeruleus (Szabo and Blier 2001) inhibits noradrenergic neurons. If we consider our previous findings (Darracq et al. 1998; Drouin et al. 2002), a decreased firing of noradrenergic neurons should induce a decreased locomotor response to *d*-amphetamine. Thus, depending on the localization of 5-HT_{2A} receptors, VTA or locus coeruleus, stimulation of 5-HT_{2A} receptors may, respectively, increase or decrease *d*-amphetamine locomotor response. In any case, our data show clearly that the chronic absence of a receptor induced by a genetic deletion may have a behavioral consequence opposite to its pharmacological acute blockade. We are not aware of any other similar example in the literature. However, Heisler and Tecott (2000) have shown that the deletion of 5-HT_{2C} receptors unmasks a pharmacological effect on 5-HT_{1B} receptors.

In conclusion, we show that 5-HT_{2A}-R KO mice are hyperresponsive to *d*-amphetamine and propose that this is due to a disinhibition of noradrenergic neurons activated by *d*-amphetamine. This increased noradrenergic response may compensate for the absence of the 5-HT_{2A} receptors located in the VTA and/or in the prefrontal cortex. Our data also indicate that repeated amphetamine treatments disrupt the inhibition of noradrenergic neurons by 5-HT_{2A} receptors thus suggesting a physiological basis of behavioral sensitization to *d*-amphetamine.

Acknowledgments We thank L. Lanfumey and M. Hamon for 5-HT_{2A}-R KO mice, S. Cotecchia for α1b-AR KO mice, J. Naudé for statistical advice, and P. Babouram for skilful technical assistance.

References

Antelman SM, Caggiula AR (1977) Norepinephrine-dopamine interactions and behavior. Science 195:646–653

Auclair A, Cotecchia S, Glowinski J, Tassin JP (2002) D-Amphetamine fails to increase extracellular dopamine levels in mice lacking alpha 1b-adrenergic receptors: relationship between functional and nonfunctional dopamine release. J Neurosci 22:9150–9154

Auclair A, Blanc G, Glowinski J, Tassin JP (2004a) Role of serotonin 2A receptors in the D-amphetamine-induced release of dopamine: comparison with previous data on alpha1b-adrenergic receptors. J Neurochem 91:318–326

Auclair A, Drouin C, Cotecchia S, Glowinski J, Tassin JP (2004b) 5-HT_{2A} and alpha1b-adrenergic receptors entirely mediate dopamine release, locomotor response and behavioural sensitization to opiates and psychostimulants. Eur J Neurosci 20:3073–3084

Besson MJ, Cheramy A, Feltz P, Glowinski J (1971) Dopamine: spontaneous and drug-induced release from the caudate nucleus in the cat. Brain Res 32:407–424

Blanc G, Trovero F, Vezina P, Herve D, Godeheu AM, Glowinski J, Tassin JP (1994) Blockade of prefronto-cortical alpha 1-adrenergic receptors prevents locomotor hyperactivity induced by subcortical D-amphetamine injection. Eur J Neurosci 6:293–298

Bortolozzi A, Diaz-Mataix L, Scorza MC, Celada P, Artigas F (2005) The activation of 5-HT receptors in prefrontal cortex enhances dopaminergic activity. J Neurochem 95:1597–1607

Cavalli A, Lattion AL, Hummler E, Nenniger M, Pedrazzini T, Aubert JF, Michel MC, Yang M, Lembo G, Vecchione C, Mostardini M, Schmidt A, Beermann F, Cotecchia S (1997) Decreased blood pressure response in mice deficient of the alpha1b-adrenergic receptor. Proc Natl Acad Sci USA 94:11589–11594

Chiang C, Aston-Jones G (1993) A 5-hydroxytryptamine2 agonist augments gamma-aminobutyric acid and excitatory amino acid inputs to noradrenergic locus coeruleus neurons. Neuroscience 54:409–420

Darracq L, Blanc G, Glowinski J, Tassin JP (1998) Importance of the noradrenaline-dopamine coupling in the locomotor activating effects of D-amphetamine. J Neurosci 18:2729–2739

Dickinson SL, Gadie B, Tulloch IF (1988) Alpha 1- and alpha 2-adrenoreceptor antagonists differentially influence locomotor and stereotyped behaviour induced by *d*-amphetamine and apomorphine in the rat. Psychopharmacology (Berl) 96:521–527

Doherty MD, Pickel VM (2000) Ultrastructural localization of the serotonin 2A receptor in dopaminergic neurons in the ventral tegmental area. Brain Res 864:176–185

Drouin C, Darracq L, Trovero F, Blanc G, Glowinski J, Cotecchia S, Tassin JP (2002) Alpha1b-adrenergic receptors control locomotor and rewarding effects of psychostimulants and opiates. J Neurosci 22:2873–2884

Fiorica-Howells E, Hen R, Gingrich J, Li Z, Gershon MD (2002) 5-HT(2A) receptors: location and functional analysis in intestines of wild-type and 5-HT(2A) knockout mice. Am J Physiol Gastrointest Liver Physiol 282:877–893

Heisler LK, Tecott LH (2000) A paradoxical locomotor response in serotonin 5-HT(2C) receptor mutant mice. J Neurosci 20:RC71

Ichikawa J, Meltzer HY (1995) DOI, a 5-HT2A/2C receptor agonist, potentiates amphetamine-induced dopamine release in rat striatum. Brain Res 698:204–208

Kelly PH, Seviour PW, Iversen SD (1975) Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res 94:507–522

Kokkinidis L, Anisman H (1978) Involvement of norepinephrine in startle arousal after acute and chronic *d*-amphetamine administration. Psychopharmacology (Berl) 59:285–292

Kokkinidis L, Anisman H (1979) Circling behavior following systemic *d*-amphetamine administration: potential noradrenergic and dopaminergic involvement. Psychopharmacology (Berl) 64:45–54

Lategan AJ, Marien MR, Colpaert FC (1990) Effects of locus coeruleus lesions on the release of endogenous dopamine in the rat nucleus accumbens and caudate nucleus as determined by intracerebral microdialysis. Brain Res 523:134–138

Moser PC, Moran PM, Frank RA, Kehne JH (1996) Reversal of amphetamine-induced behaviours by MDL 100,907, a selective 5-HT2A antagonist. Behav Brain Res 73:163–167

Ogren SO, Archer T, Johansson C (1983) Evidence for a selective brain noradrenergic involvement in the locomotor stimulant effects of amphetamine in the rat. Neurosci Lett 43:327–331

O'Neill MF, Heron-Maxwell CL, Shaw G (1999) 5-HT_2 receptor antagonism reduces hyperactivity induced by amphetamine, cocaine, and MK-801 but not D1 agonist C-APB. Pharmacol Biochem Behav 63:237–243

Pazos A, Cortes R, Palacios JM (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2 receptors. Brain Res 346:231–249

Pijnenburg AJ, Honig WM, Van Rossum JM (1975) Inhibition of *d*-amphetamine-induced locomotor activity by injection of haloperidol into the nucleus accumbens of the rat. Psychopharmacologia 41:87–95

Porras G, Di Matteo V, Fracasso C, Lucas G, De Deurwaerdere P, Caccia S, Esposito E, Spampinato U (2002) 5-HT2A and 5-HT2C/2B receptor subtypes modulate dopamine release induced in vivo by amphetamine and morphine in both the rat nucleus accumbens and striatum. Neuropsychopharmacology 26:311–324

Rinaldi-Carmona M, Congy C, Santucci V, Simiand J, Gautret B, Neliat G, Labeeuw B, Le Fur G, Soubrie P, Breliere JC (1992) Biochemical and pharmacological properties of SR 46349B, a new potent and selective 5-hydroxytryptamine2 receptor antagonist. J Pharmacol Exp Ther 262:759–768

Salomon L, Lanteri C, Glowinski J, Tassin JP (2006) Behavioral sensitization to amphetamine results from an uncoupling between noradrenergic and serotonergic neurons. Proc Natl Acad Sci USA 103:7476–7481

Shi WX, Pun CL, Zhang XX, Jones MD, Bunney BS (2000) Dual effects of D-amphetamine on dopamine neurons mediated by dopamine and nondopamine receptors. J Neurosci 20:3504–3511

Snoddy AM, Tessel RE (1985) Prazosin: effect on psychomotor-stimulant cues and locomotor activity in mice. Eur J Pharmacol 116:221–228

Sorensen SM, Kehne JH, Fadayel GM, Humphreys TM, Ketteler HJ, Sullivan CK, Taylor VL, Schmidt CJ (1993) Characterization of the 5-HT2 receptor antagonist MDL 100907 as a putative atypical antipsychotic: behavioral, electrophysiological and neurochemical studies. J Pharmacol Exp Ther 266:684–691

Szabo ST, Blier P (2001) Functional and pharmacological characterization of the modulatory role of serotonin on the firing activity of locus coeruleus norepinephrine neurons. Brain Res 922:9–20

Szabo ST, Blier P (2002) Effects of serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibition plus 5-HT(2A) receptor antagonism on the firing activity of norepinephrine neurons. J Pharmacol Exp Ther 302:983–991

Taghzouti K, Simon H, Herve D, Blanc G, Studler JM, Glowinski J, LeMoal M, Tassin JP (1988) Behavioural deficits induced by an electrolytic lesion of the rat ventral mesencephalic tegmentum are

corrected by a superimposed lesion of the dorsal noradrenergic system. Brain Res 440:172–176

Tassin JP, Studler JM, Herve D, Blanc G, Glowinski J (1986) Contribution of noradrenergic neurons to the regulation of dopaminergic (D1) receptor denervation supersensitivity in rat prefrontal cortex. J Neurochem 46:243–248

Von Voigtlander PF, Moore KE (1973) Involvement of nigro-striatal neurons in the in vivo release of dopamine by amphetamine, amantadine and tyramine. J Pharmacol Exp Ther 184:542–552

Weisstaub NV, Zhou M, Lira A, Lambe E, Gonzalez-Maeso J, Hornung JP, Sibille E, Underwood M, Itohara S, Dauer WT, Ansorge MS, Morelli E, Mann JJ, Toth M, Aghajanian G, Sealfon SC, Hen R, Gingrich JA (2006) Cortical 5-HT2A signaling modulates anxiety-like behaviours in mice. Science 313:536–540