

Dopamine D₁ receptors and adenosine A₁ receptors in the rat nucleus accumbens regulate motor activity but not prepulse inhibition

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Abstract

Locomotor activity and sensorimotor gating (measured as prepulse inhibition of startle) are regulated by mesoaccumbal dopamine. Recent evidence indicated antagonistic interactions between adenosine A₁ receptors and dopamine D₁ receptors, as well as between adenosine A₂ receptors and dopamine D₂ receptors in the nucleus accumbens. Therefore, it is conceivable that accumbal dopamine and adenosine are both involved in the regulation of prepulse inhibition and locomotion. We tested whether accumbal adenosine A₁ and dopamine D₁ receptors control locomotor activity and prepulse inhibition using the following four treatments. (1) Injections of the selective adenosine A₁ receptor agonist *N*⁶-cyclopentanyladenosine (CPA 1.5 and 3 µg/µl per side) into the nucleus accumbens. (2) Stimulation of the ventral tegmental area by local infusion of the GABA_A receptor antagonist picrotoxin (25–100 ng/0.5 µl bilaterally). (3) Picrotoxin injections into the ventral tegmental area (100 ng/0.5 µl) and simultaneous bilateral injections of CPA (3 µg/µl per side) into the nucleus accumbens. (4) Injections of the selective dopamine D₁ receptor antagonist SCH 23390 (3 µg/0.5 µl per side) into the nucleus accumbens and ventral tegmental area stimulation by picrotoxin. Intra-accumbal CPA infusion reduced locomotor activity but had no effect on prepulse inhibition. Picrotoxin stimulation of the ventral tegmental area increased locomotor activity which was antagonized by co-administration of CPA or SCH 23390 into the nucleus accumbens. An enhancement of prepulse inhibition was observed after stimulation of the ventral tegmental area and co-administration of SCH 23390 into the nucleus accumbens. These findings demonstrate that adenosine A₁ and dopamine D₁ receptors are involved in the regulation of locomotor activity mediated by the mesoaccumbal dopamine system. The finding that locomotor effects induced by stimulation of the mesoaccumbal dopamine system were not accompanied by a prepulse inhibition-deficit suggests a dissociation of the neuronal substrates involved in the control of locomotion and the regulation of sensorimotor gating. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

The mesoaccumbal dopamine system plays an important role in the regulation of adaptive behavior. For example, locomotor activity is controlled by the dopaminergic projection from the ventral tegmental area to the nucleus accumbens (Mogenson et al., 1993). Prepulse inhibition of startle is normally observed when the startling stimulus is preceded by a weak prepulse presented some 100 ms before the startling stimulus (Hoffman and Ison, 1980) and serves as an operational measure of sensorimotor gating mechanisms which normally inhibit inadvertent motor responses to sensory stimuli. Prepulse inhibition is reduced after administra-

tion of dopamine receptor agonists into the nucleus accumbens, and this effect has been interpreted as a result of tuning down of sensorimotor gating under conditions of enhanced exploratory behavior (Swerdlow et al., 1993).

While dopamine is the most prominent neurotransmitter involved in the control of prepulse inhibition and locomotor activity, there is much evidence that neuromodulators like adenosine are also important for the regulation of prepulse inhibition and locomotor activity presumably by interacting with dopamine (Ferré, 1997; Koch and Hauber, 1998). Adenosine modulates central neurotransmission through its action on four different G-protein-coupled receptors termed A₁, A_{2A}, A_{2B} and A₃ (Ralevic and Burnstock, 1998). There are antagonistic interactions between dopamine D₂ and adenosine A_{2A} receptors, as well as between dopamine D₁ and adenosine A₁ receptors. Accordingly, stimulation of

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adenosine A₁ or A_{2A} receptors inhibits dopamine D₁ or D₂ receptor-mediated effects, respectively (see Ferré, 1997 for review; Ginés et al., 2000). Adenosine A₁ and A_{2A} receptor agonists have been shown to produce a marked decrease in locomotor activity and reduce hyperactivity induced by dopamine receptor stimulation in rats. On the other hand, unselective adenosine receptor antagonists like the methylxanthines caffeine and theophylline enhance locomotor activity and reverse motor hypoactivity after acute dopamine depletion or systemic administration of dopamine receptor antagonists (see Ferré, 1997 for review). Furthermore, adenosine receptor antagonists potentiate hyperactivity elicited by dopamine receptor stimulation (e.g. Popoli et al., 1996). Previous studies mainly used locomotor activity as the behavioral parameter to investigate adenosine–dopamine interactions, but recent behavioral studies showed that interactions between adenosine and dopamine also regulate prepulse inhibition. Systemical application of the A₁ receptor agonist N⁶-cyclopentyladenosine (CPA) (Koch and Hauber, 1998) and intra-accumbal infusion of the A_{2A} receptor agonist CGS 21680 (Hauber and Koch, 1997) reversed prepulse inhibition deficits induced by apomorphine. These results indicate that antagonistic interactions between adenosine and dopamine probably in the nucleus accumbens play a critical role in the regulation of sensorimotor gating.

Since the nucleus accumbens is a key structure for the control of both locomotor activity and prepulse inhibition, the present study compared the effects of a neurochemical stimulation of the mesoaccumbal dopamine system on locomotor activity and on prepulse inhibition and investigated whether these effects are modulated by adenosine A₁ receptors and dopamine D₁ receptors in the nucleus accumbens. In the first experiment, we tested the effect of the adenosine A₁ receptor agonist CPA infused into the nucleus accumbens on locomotor activity and prepulse inhibition. In further experiments, we investigated whether stimulation of the mesoaccumbal dopamine system by injections of the GABA_A receptor antagonist picrotoxin into the ventral tegmental area affected locomotor activity and prepulse inhibition, and whether intra-accumbal co-administration of CPA and of the dopamine D₁ receptor antagonist SCH 23390 could reverse the effects of picrotoxin.

2. Materials and methods

2.1. Animals

A total of 63 naive male Sprague–Dawley rats (Charles River, Sulzfeld, Germany) weighing 200–310 g at the time of surgery were housed in groups of five to six rats per cage in a temperature- and humidity-controlled colony room under a 12:12 h light/dark cycle (lights on at 07:00). They were fed with 12 g of standard rat chow/animal/day, and tap water was available ad libitum. The rats were handled daily

before and after surgery. The experiments were performed in accordance with international ethical guidelines for the care and use of animals for experiments and were approved by the local council of animal care (Regierungspräsidium Tübingen, ZP 4/96). A total of 18 rats had misplaced injections, so the data were not used for statistical analysis.

2.2. Surgery

Rats were anesthetized with ketavet/xylazine (9:1, 100 mg/kg i.p.) and placed into a stereotaxic frame. The skull was exposed and stainless steel guide cannulae (0.7-mm diameter) were implanted bilaterally aiming at the nucleus accumbens (toothbar 5 mm above the interaural plane; distance from Bregma: rostrocaudal 3.4 mm, mediolateral 1.5 mm, dorsoventral 7.2 mm; coordinates after Pellegrino et al., 1979) and the ventral tegmental area (toothbar—3.3 mm below the interaural plane; distance from Bregma: rostrocaudal 5.3 mm, mediolateral 1.4 mm, dorsoventral 8.0 mm; coordinates after Paxinos and Watson, 1997). For the ventral tegmental area-cannulae, a mediolateral angle of 6° was used in order to avoid damage of the medial blood sinus. The guide cannulae were fixed to the skull with acrylic cement (Kulzer, Wehrheim, Germany) and three anchoring screws. After surgery, the cannulae were fitted with stylets in order to maintain patency. The behavioral tests commenced when the animals had recovered from surgery, which was 5 days after surgery.

2.3. Apparatus and testing procedure

2.3.1. Startle measurements

The acoustic startle response was measured with two identical stabilimeter devices in two sound-attenuated chambers (100 × 80 × 60 cm³ each). For the tests, the rats were placed in wire mesh cages with steel floors (20 × 10 × 12 cm³) which were fixed onto piezoelectric accelerometers. Movements of the rats caused changes of the stabilimeter voltage output that were amplified, digitized and analyzed by a PC. The acoustic startle response magnitude was computed as the difference of the peak-to-peak voltage output of the accelerometer 80 ms before and 80 ms after the onset of the startling stimulus. Acoustic stimuli were generated by a function-synthesizer (Hortmann, Neckartenzlingen, Germany) and delivered through loudspeakers mounted 40 cm away from the test cages. A continuous white background noise (55-dB sound pressure level, SPL) was presented throughout the test session. Intensity measurements were done with a 0.5-in. condenser microphone and a measuring amplifier (Brüel and Kjaer, Copenhagen, Denmark) after bandpass (0.25–80 kHz) filtering.

After a 5-min acclimatization period, during which the rats received no stimuli except the background noise, the test session began with an initial startle stimulus followed by five different trial types presented in a random order: (1) acoustic startle stimulus alone (white noise, 100-dB SPL re

2×10^{-5} N/m², 20 ms duration), (2) acoustic startle stimulus preceded by a 70-dB SPL prepulse (10 kHz, 20 ms duration including rise/fall times of 0.4 ms, 100 ms before onset of the pulse), (3) acoustic startle stimulus preceded by a 60-dB SPL prepulse (10 kHz, 20 ms duration including rise/fall times of 0.4 ms, 100 ms before onset of the startle stimulus), (4) prepulse (70 dB) alone and (5) no stimulus. Test duration was 35 min and interstimulus interval was 30 s.

2.3.2. Activity measurements

Locomotor activity was measured in an open field (80-cm diameter, 25-cm height) made of gray plastic. The open field was divided into eight subfields. A continuous background noise (50 dB) was presented throughout the test sessions. At the beginning of the test sessions, each rat was placed in the middle of the open field and horizontal activity (line crossing of all four legs) and vertical activity (rearings) were quantified over 35 min. Behavioral parameters were evaluated online by an observer via a CCD camera.

2.3.3. Testing procedure

Four experiments were performed with different groups of rats as described below. In all experiments, rats received microinjections via stainless steel cannulae (0.4 mm in diameter) into the nucleus accumbens or/and ventral tegmental area. Each test day was followed by one drug/test-free day. A counterbalanced Latin square design was used, i.e. each rat received one of four different treatments per test day in a random order. So each rat of a given experiment received each drug and was tested both for locomotor activity and prepulse inhibition (except experiment 3). Within subject comparisons were made.

In the first experiment, rats ($n=10$) received microinjections into the nucleus accumbens of either saline or the adenosine A₁ receptor agonist CPA (1.5 and 3 $\mu\text{g}/\mu\text{l}$ for prepulse inhibition testings; 3 $\mu\text{g}/\mu\text{l}$ for locomotor activity) and were tested for prepulse inhibition and locomotor activity immediately afterwards. In the second experiment, another group of rats ($n=16$) received microinjections of either saline or the GABA_A receptor antagonist picrotoxin (25, 50 and 100 ng/0.5 μl) into the ventral tegmental area and were tested for prepulse inhibition and locomotor activity. In the following experiment, a third group of rats ($n=8$) received combinations of microinjections of picrotoxin (100 ng/0.5 μl) or saline into the ventral tegmental area, and CPA (3 $\mu\text{g}/\mu\text{l}$ aqua dest) or saline into the nucleus accumbens and were tested for locomotor activity. CPA was given first and picrotoxin was infused immediately afterwards. In the fourth experiment, rats received combinations of microinjections of picrotoxin (100 ng/0.5 μl) or saline into the ventral tegmental area, and SCH 23390 (3 $\mu\text{g}/0.5 \mu\text{l}$) or saline into the nucleus accumbens and were tested for prepulse inhibition ($n=8$) or locomotor activity ($n=11$, 8 rats from the prepulse inhibition test, plus 3 new rats). SCH

23390 was given first and picrotoxin was infused immediately afterwards. All rats were tested immediately after the microinjections. For each rat of each experiment was one drug-free day between testing days. The drugs were administered bilaterally with 2- or 4- μl syringes (SGE, Weiterstadt, Germany), each connected to an injection cannula by flexible PVC tubes. Infusion rate was 0.1 $\mu\text{l}/20$ s and the injection cannulae remained in the brain for 60 s after completing the injection. The interval between experiments 1 and 4 was about 1 year.

2.4. Drugs

CPA (*N*⁶-cyclopentanyladenosine, RBI, Massachusetts, USA; pH 7.2), picrotoxin (Sigma, Steinheim; pH 7.0) and SCH 23390 (Sigma; pH 7.0) were dissolved in distilled water and were administered in a volume of 0.5 μl (for picrotoxin and SCH 23390) or 1.0 μl (for CPA, because of its low solubility in higher concentrations).

2.5. Histology

After the tests, the rats were sacrificed by an overdose of pentobarbital and their brains were removed and fixed in 8% paraformaldehyde and 20% sucrose in 0.1 M phosphate buffer. Frontal sections (50 μm) were cut on a freezing microtome and Nissl stained with Thionin. The injection sites were localized and the extent of tissue lesions due to cannulation was examined under a light microscope. The injection sites were drawn onto plates taken from a rat brain atlas (Paxinos and Watson, 1997).

2.6. Data analysis

The acoustic startle response amplitudes of each different trial type were averaged and are presented as mean standard \pm errors (\pm S.E.M.). Prepulse inhibition was the percent decrease of the acoustic startle response following a startle stimulus preceded by a prepulse compared to the acoustic startle response without prepulse ($100 \times (\text{mean acoustic startle response amplitude on pulse-alone trials} - \text{mean acoustic startle response amplitude on prepulse-pulse trials}) / \text{mean acoustic startle response amplitude on pulse-alone trials}$). The treatment effects on prepulse inhibition and pulse-alone acoustic startle response amplitudes were evaluated using analyses of variance (ANOVA) followed by post hoc Tukey's *t*-test for pairwise comparison. A value of $P < 0.05$ was considered to represent a significant effect.

Rearings and line crossings were averaged and are presented as mean \pm S.E.M. The treatment effects on rearings and line crossings of the first 10 min were analyzed by Student *t*-tests and by repeated measures ANOVA after square-root transformation, followed by post hoc Tukey's *t*-test for pairwise comparison. A value of $P < 0.05$ was considered to represent a significant effect.

3. Results

3.1. Histology

The injection sites of CPA and SCH 23390 were localized in the core or shell region of the nucleus accumbens and the injection sites of picrotoxin were localized in the ventral tegmental area in all rats (Fig. 1).

3.2. Experiment 1: effects of the adenosine A_1 receptor agonist CPA on locomotor activity and prepulse inhibition

CPA injection into the nucleus accumbens significantly reduced both line crossings ($t=10.519$, $P<0.01$; Fig. 2) and rearings ($t=9.946$, $P<0.01$; $n=10$, Fig. 2). There were no significant drug-effects on prepulse inhibition (60 and 70 dB) and acoustic startle response amplitude after micro-injections of CPA into the nucleus accumbens (ANOVAs: F 's <1 , P 's >0.67 ; $n=10$; Table 1), no significant dose \times prepulse intensity interaction (ANOVA: $F(2,45)=0.108$, $P=0.898$), but a significant effect of prepulse intensity (ANOVA: $F(1,45)=6.023$, $P<0.05$).

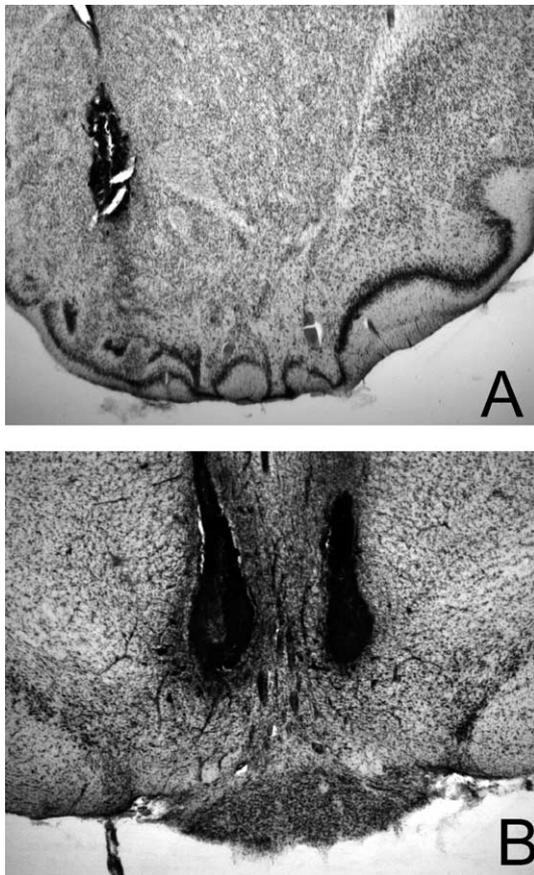


Fig. 1. Photomicrographies showing typical injection sites (A) into the nucleus accumbens and (B) into the ventral tegmental area.

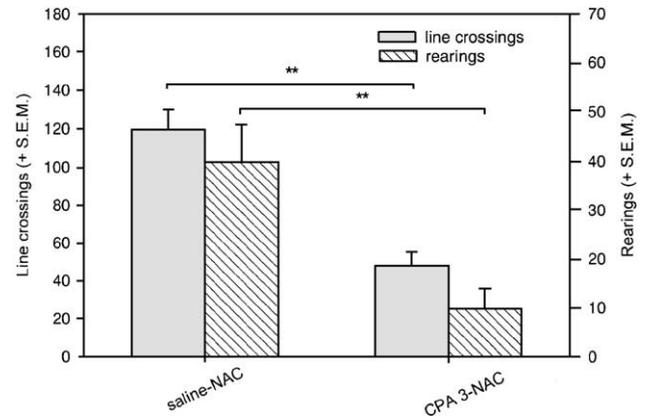


Fig. 2. Numbers of line crossings and rearings (means \pm S.E.M.) after intra-accumbal administration of 3 μ g CPA or saline. CPA significantly reduced the number of line crossings and rearings of rats in the open field. ** $P<0.01$ (Student's t -test).

3.3. Experiment 2: effects of the $GABA_A$ receptor antagonist picrotoxin in the ventral tegmental area on locomotor activity and prepulse inhibition

Picrotoxin injection into the ventral tegmental area dose-dependently enhanced the number of line crossings (ANOVA: $F(3,45)=13.12$, $P<0.01$, Tukey's t -test: $P<0.01$; $n=16$, Table 2) but not rearings (ANOVA: $F(3,45)=0.823$, $P=0.26$, Tukey's t -test: $P=0.5$; $n=16$, Table 2). No significant treatment effects on prepulse inhibition (60 and 70 dB) and on the acoustic startle response (ANOVAs: F 's <2.0 , P 's >0.14 ; $n=16$) were found after microinjections of picrotoxin into the ventral tegmental area (Table 2). There were no significant dose \times prepulse intensity interaction (ANOVA: $F(3,105)>0.64$, $P>0.59$) and no significant effect of prepulse intensity (ANOVA: $F(1,105)>2.82$, $P>0.09$) after microinjections of picrotoxin into the ventral tegmental area.

3.4. Experiment 3: effects of the $GABA_A$ receptor antagonist picrotoxin in the ventral tegmental area and the A_1 receptor agonist CPA in the nucleus accumbens on locomotor activity

CPA injection into the nucleus accumbens significantly reduced both line crossings (for injection into the nucleus

Table 1
Effects of CPA injections into the nucleus accumbens on prepulse inhibition and acoustic startle response

	Saline	1.5 μ g CPA	3 μ g CPA
Mean % prepulse inhibition (60 dB) \pm S.E.M.	40.0 \pm 6.4	43.2 \pm 7.7	37.0 \pm 8.7
Mean % prepulse inhibition (70 dB) \pm S.E.M.	59.3 \pm 8.3	56.0 \pm 7.8	50.7 \pm 9.5
Mean % prepulse inhibition (60 and 70 dB) \pm S.E.M.	49.6 \pm 5.6	49.6 \pm 5.5	43.9 \pm 6.5
Mean acoustic startle response \pm S.E.M.	101.3 \pm 29.5	104.8 \pm 15.2	105.5 \pm 20.0

Table 2
Effects of picrotoxin-injections into the ventral tegmental area on prepulse inhibition, acoustic startle response and locomotor activity

	Saline	25 ng PTX	50 ng PTX	100 ng PTX
Mean % prepulse inhibition (60 dB) ± S.E.M.	30.8 ± 4.1	31.0 ± 4.8	37.9 ± 4.8	38.9 ± 8.4
Mean % prepulse inhibition (70 dB) ± S.E.M.	30.2 ± 6.7	43.0 ± 5.8	41.2 ± 6.9	50.7 ± 8.2
Mean % prepulse inhibition ± S.E.M.	30.5 ± 3.9	37.0 ± 3.8	39.6 ± 4.1	44.4 ± 5.9
Mean acoustic startle response	178.9 ± 26.0	180.2 ± 27.1	174.9 ± 29.0	144.2 ± 18.6
Line crossings ± S.E.M.	101.8 ± 9.7	105.8 ± 12.7	123.9 ± 9.9	178.8 ± 14.1
Rearings ± S.E.M.	29.5 ± 3.3	29.1 ± 3.9	32.6 ± 5.1	37.1 ± 4.4

accumbens and the ventral tegmental area, ANOVA: F 's>12.28, P <0.01; Tukey's t -test: P <0.01; n =8) and rearings (injection into the nucleus accumbens, ANOVA: $F(1,21)=24.93$, P <0.01 and injection into the ventral tegmental area, ANOVA: $F(1,21)=0.016$, P =0.902, Tukey's t -test: P <0.01; n =8, Fig. 3). Microinjection of picrotoxin into the ventral tegmental area significantly enhanced line crossings (Tukey's t -test: P <0.05) but not rearings (Tukey's t -test: P =1) and the concomitant CPA injection into the nucleus accumbens significantly antagonized this locomotor-stimulating effect (Tukey's t -test: P <0.01). ANOVA revealed no significant drug × drug interaction (ANOVAs: F 's>0.009, P >0.16).

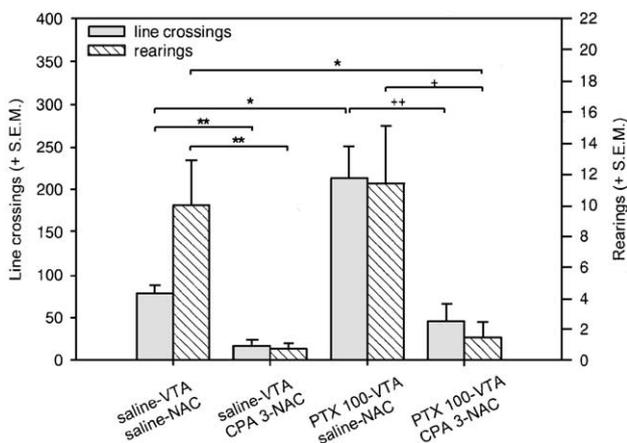


Fig. 3. Numbers of line crossings and rearings (means ± S.E.M.) after infusion of 3 µg CPA into the nucleus accumbens (CPA 3-NAC) and 100 ng picrotoxin into the ventral tegmental area (PTX 100-VTA). CPA significantly reduced the line crossing enhancing effect of picrotoxin; ** P <0.01; * P <0.05 compared to the respective saline infusion; +++ P <0.01; + P <0.05 compared to picrotoxin (ANOVA followed by Tukey's t -test).

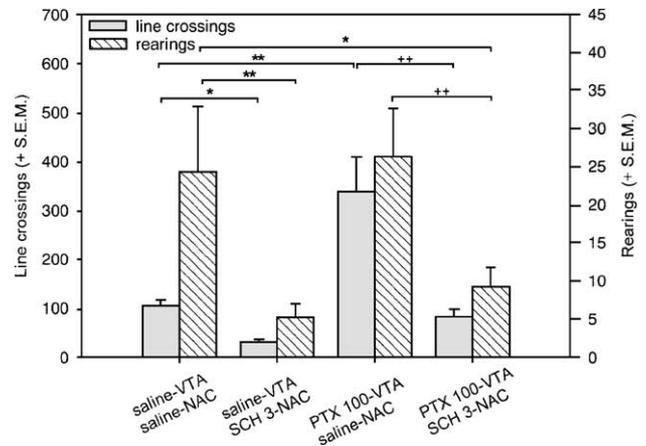


Fig. 4. Number of line crossings and rearings (means ± S.E.M.) after injections of 3 µg SCH 23390 (SCH 3-NAC) into the nucleus accumbens and 100 ng picrotoxin into the ventral tegmental area (PTX 100-VTA). Intra-accumbal injection of SCH 23390 significantly reduced the enhancing effect on line crossings of intraventricular injection of picrotoxin; ** P <0.01; * P <0.05 compared to the respective saline infusion; +++ P <0.01; + P <0.05 compared to picrotoxin (ANOVA followed by Tukey's t -test).

3.5. Experiment 4: effects of the GABA_A receptor antagonist picrotoxin and the dopamine D₁ receptor antagonist SCH 23390 on locomotor activity and prepulse inhibition

Injection of SCH 23390 into the nucleus accumbens significantly reduced both line crossings (ANOVA: F 's>22.32, P <0.01, Tukey's t -test: P <0.05; n =11, Fig. 4) and rearings (ANOVA: F 's>2.0, P <0.01, Tukey's t -test: P <0.01; n =11, Fig. 4). Picrotoxin injected into the ventral tegmental area significantly enhanced line crossings

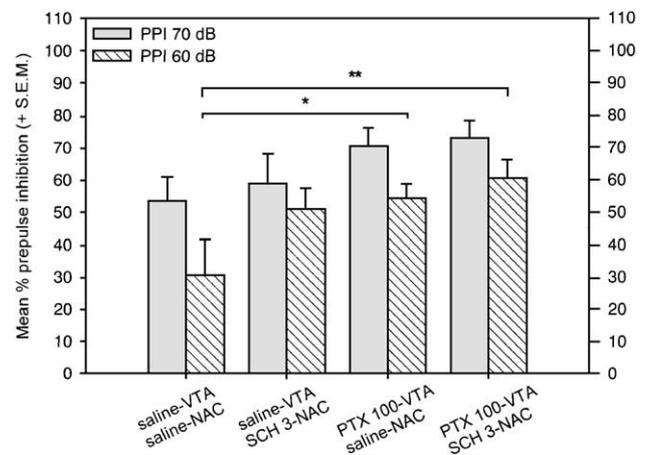


Fig. 5. Prepulse inhibition with prepulses of 60 or 70 dB (means ± S.E.M.) after injections of 3 µg SCH 23390 or saline into the nucleus accumbens and 100 ng picrotoxin or saline into the ventral tegmental area. There was no significant effect on prepulse inhibition with prepulses of 70 dB. Prepulse inhibition with prepulses of 60 dB was significantly enhanced after simultaneous injection of either intraventricular picrotoxin and intranucleus accumbens saline or intraventricular picrotoxin and intranucleus accumbens SCH 23390; ** P <0.01, * P <0.05 (ANOVA followed by Tukey's t -test).

(Tukey's *t*-test: $P < 0.01$) but not rearings (Tukey's *t*-test: $P = 0.924$) and SCH 23390 significantly attenuated this effects (for line crossings: Tukey's *t*-test: $P < 0.01$; for rearings: Tukey's *t*-test: $P < 0.01$).

There was no significant effect on prepulse inhibition at 60 dB prepulse intensity after injection of SCH 23390 into the nucleus accumbens (ANOVA: $F(1,21) = 5.725$, $P < 0.05$, Tukey's *t*-test: $P = 0.076$; $n = 8$, Fig. 5), but microinjections of picrotoxin into the ventral tegmental area as well as a combination of picrotoxin infusion and SCH 23390 infusion significantly enhanced prepulse inhibition at 60 dB prepulses (ANOVA: $F(1,21) = 8.909$, $P < 0.01$; Tukey's *t*-test: $P < 0.05$, combination: $P < 0.01$; $n = 8$). No significant effects on prepulse inhibition at 70 dB prepulses were seen after either microinjection of SCH 23390 into the nucleus accumbens or picrotoxin into the ventral tegmental area or a combination of SCH 23390 and picrotoxin (for injections into the nucleus accumbens, ANOVA: $F(1,21) = 0.0502$, $P = 0.487$ and for injections into the ventral tegmental area, ANOVA: $F(1,21) = 7.551$, $P = 0.012$; Tukey's *t*-test: $P =$ no significant data; $n = 8$). ANOVA revealed no significant dose \times prepulse intensity interaction for both picrotoxin and SCH 23390 (ANOVAs: F 's > 0.014 , P 's > 0.3). There was no effect on acoustic startle response amplitude after microinjection of SCH 23390 into the nucleus accumbens (for injections into the nucleus accumbens, ANOVA: $F(1,21) = 1.101$, $P = 0.306$ and for injections into the ventral tegmental area, ANOVA: $F(1,21) = 11.632$, $P < 0.01$, Tukey's *t*-test: $P = 0.629$; $n = 8$). Injection of picrotoxin into the ventral tegmental area and combined injection of SCH 23390 and picrotoxin both enhanced baseline acoustic startle response amplitude by

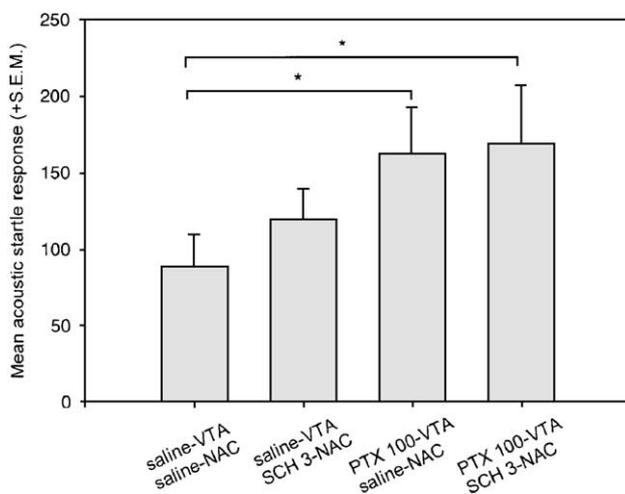


Fig. 6. Acoustic startle reaction (means \pm S.E.M.) after injections of 3 μ g SCH 23390 or saline into the nucleus accumbens and 100 ng picrotoxin or saline into the ventral tegmental area. There was no significant effect on prepulse inhibition with prepulses of 70 dB. Acoustic startle reaction was significantly enhanced after simultaneous injection of either intraventral tegmental area picrotoxin and intranucleus accumbens saline or intraventral tegmental area picrotoxin and intranucleus accumbens SCH 23390; ** $P < 0.01$, * $P < 0.05$ compared to the respective saline infusion (ANOVA followed by Tukey's *t*-test).

82.5% and 90.4%, respectively (Tukey's *t*-test: $P < 0.05$; Fig. 6). ANOVA revealed no significant drug \times drug interaction (for prepulse inhibition and acoustic startle response, ANOVA: F 's > 0.062 , P 's > 0.22 ; for line crossings and rearings, ANOVA: F 's > 0.288 , P 's > 0.062).

4. Discussion

The present findings indicate that activation of adenosine A_1 receptors or blockade of dopamine in the nucleus accumbens results in a marked decrease of locomotor activity. In line with other studies (Meyer, 1993; Swanson et al., 1997), we found that the dopamine receptor antagonist SCH 23390 reduces locomotor activity, suggesting that accumbal dopamine D_1 receptors also play an important role in the modulation of locomotor activity. Also, the adenosine A_1 receptor agonist CPA reduces locomotor activity in our study and this goes in line with previous studies showing that the adenosine A_1 receptor agonist CPA reduces locomotor activity after either systemical or intrastriatal injections (e.g. Ferré et al., 1994; Popoli et al., 1994; Brockwell and Beninger, 1996).

The exact mechanism underlying the CPA-induced locomotor inhibition is not clear. One possibility is that inhibition of locomotor activity caused by CPA could be mediated by intramembrane interactions between adenosine A_1 and dopamine D_1 receptors (Ferré et al., 1994; Turgeon et al., 1996; Ginés et al., 2000) in the nucleus accumbens. However, we cannot exclude that the effects of CPA are mediated by a stimulation of presynaptic adenosine A_1 receptors, thereby blocking dopamine and glutamate release (Ferré, 1997).

Prepulse inhibition of the acoustic startle response was not affected by injections of the adenosine A_1 receptor agonist CPA into the nucleus accumbens. Koch and Hauber (1998) showed that systemic administration of 1.5 mg/kg of the adenosine A_1 receptor agonist CPA enhanced prepulse inhibition and the prepulse inhibition disruptive effect of a combination of low doses of apomorphine and of the adenosine receptor antagonist theophylline was reversed by CPA. However, Sills et al. (1999) reported no effect of systemic administration of 0.5 mg/kg CPA on prepulse inhibition, suggesting that a relatively high dose of CPA is required for an effect on prepulse inhibition. Based on a local infusion study, Hauber and Koch (1997) suggested that the nucleus accumbens mediate the effects of adenosine receptor stimulation on prepulse inhibition by an interaction of adenosine and dopamine receptors. However, the lack of effect of intra-accumbal administration of CPA on prepulse inhibition shown in the present study suggests that the prepulse inhibition-enhancing effect of systemically administered CPA (Koch and Hauber, 1998) is not mediated by the nucleus accumbens.

We also showed in the present study that picrotoxin-induced blockade of GABA_A receptors in the ventral tegmental area enhances line crossings but not rearings. The

increase of locomotion is in accordance with the results of Mogenson et al. (1979). In this study, the ventral tegmental area stimulation by picrotoxin had a locomotor-enhancing effect, so it was suggested that dopaminergic ventral tegmental area neurons are under tonic GABAergic inhibition, and that disinhibition of the ventral tegmental area by picrotoxin leads to dopamine release in the nucleus accumbens. Other previous studies confirmed that mesoaccumbal dopamine plays an important role in locomotor activity since electrical stimulation of the ventral tegmental area and injections of dopamine or dopamine receptor agonists into the nucleus accumbens enhance locomotor activity (Kelley et al., 1989; Ott and Mandel, 1995; summarized in Mogenson et al., 1993). We therefore suggest that the increased locomotor activity shown in the present study was due to a disinhibition of mesoaccumbal dopamine neurons.

Our data show that activation of adenosine A₁ receptors or blockade of dopamine D₁ receptors within the nucleus accumbens reduced the locomotor activation induced by the ventral tegmental area stimulation with picrotoxin. There is evidence that the dopamine D₁ receptors are the main target of mesoaccumbal dopamine relevant for locomotion because other studies found that dopamine D₁ receptor agonists and antagonists had stronger effects on locomotor activity than dopamine D₂ receptor agonists and antagonists (Swanson et al., 1997; Dreher and Jackson, 1998). Blockade of accumbal dopamine D₁ receptors also strongly reduced instrumental responding for reward (Koch et al., 2000).

Interestingly, our data show that intraventral tegmental area infusion of picrotoxin in a dose which induced a prominent increase of locomotor activity had no significant effects on prepulse inhibition, suggesting that a relatively strong stimulation of the ventral tegmental area probably resulting in an increased synaptic dopamine release in the nucleus accumbens (Mogenson et al., 1993) did not reduce prepulse inhibition. These data seem to be at variance with the general notion that overactivity of the mesoaccumbal dopamine system results in reduced prepulse inhibition (Swerdlow et al., 1992; Zhang et al., 2000). Zhang et al. (2000) showed that there is an inverse relationship between dopamine overflow in the nucleus accumbens and the amount of prepulse inhibition. They showed that equimolar doses of amphetamine and cocaine were not equally effective in reducing prepulse inhibition and enhancing dopamine overflow. Amphetamine caused a marked increase in dopamine overflow in the nucleus accumbens and there was a close relationship over time with the reduction of prepulse inhibition at higher dopamine concentrations. Administration of cocaine at equimolar dose also caused an increase in dopamine overflow in the nucleus accumbens, but this increase was less pronounced in magnitude compared to that of amphetamine. The moderate overflow of dopamine in the nucleus accumbens after cocaine administration caused no reduction of prepulse inhibition. Therefore, dopamine in the nucleus accumbens must reach very high concentrations before leading to a reduced prepulse inhibition. Amphet-

amine and cocaine are indirect dopamine agonists acting by blocking the dopamine re-uptake into dopamine terminals. Therefore, they may induce higher dopamine concentrations at the synapse than picrotoxin injected into the ventral tegmental area does.

Moreover, Zhang et al. (1995) also showed that pertussis toxin injected into the ventral tegmental area, which blocks GABA receptors indirectly, had no effect on prepulse inhibition per se but reduced prepulse inhibition in rats treated with a combination of pertussis toxin with a systemically applied low dose of amphetamine. These findings, together with our data, suggest that only a very high concentration of dopamine in the nucleus accumbens reduces prepulse inhibition.

Interestingly, we found that co-microinfusions of picrotoxin into the ventral tegmental area and saline into the nucleus accumbens significantly enhanced prepulse inhibition with a prepulse intensity of 60 dB, but not of 70 dB. There was a trend of an enhanced prepulse inhibition with prepulses of 70 dB. Interestingly, prepulse inhibition was significantly enhanced when SCH 23390 was infused into the nucleus accumbens during stimulation of the ventral tegmental area, but not after administration of SCH 23390 alone. Only a tendency of enhancement of prepulse inhibition with a prepulse intensity of 60 dB was seen after injections of SCH 23390 into the nucleus accumbens and saline into the ventral tegmental area. The interpretation of these results is difficult since the stimulation of the ventral tegmental area probably increases dopamine release in the nucleus accumbens. In other studies, a dopamine release in the nucleus accumbens was accompanied by a reduction of prepulse inhibition (Swerdlow et al., 1992; Zhang et al., 2000), while injections of dopamine D₁ receptor antagonists have been reported to enhance prepulse inhibition (Schwarzkopf et al., 1993; Hoffman and Donovan, 1994; Depoortere et al., 1997). The phenomenon of an enhanced prepulse inhibition after increased dopamine was described in a few recent studies (Swerdlow et al., 2001; Martin-Iverson and Else, 2000). In both studies, different stimulus parameters (e.g. different prepulse intensities or different prepulse intervals) were used. The conclusion of these studies was that different dopamine agonists not only disrupt prepulse inhibition, but also increase prepulse inhibition, depending upon the stimulus parameters. They found that weak prepulses, relatively long prepulse intervals or very short prepulse intervals lead to an enhancement of prepulse inhibition with dopamine agonists. In line with Swerdlow et al. (2001), we used prepulse intervals of 100 ms, which is a relatively long interval with prepulse intensities, and the weaker prepulse intensity leads to enhancement of prepulse inhibition.

Our results also showed that stimulation of the ventral tegmental area leads to an enhancement of the acoustic startle response magnitude (experiment 4), and the injection of SCH 23390 into the nucleus accumbens has no effect on this enhancement. Previous studies have shown that electrical stimulation of the ventral tegmental area leads to an

enhancement of the acoustic startle response (Borowski and Kokkinidis, 1996) since electrical stimulation of the ventral tegmental area also leads to activation of the amygdala (Gerlowitz and Kokkinidis, 1999). Therefore, we suggest that the acoustic startle response-enhancing effect in our study was due to an indirect activation of the amygdala, and that the treatment, therefore, of the nucleus accumbens with SCH 23390 did not reverse the potentiation of the acoustic startle response.

One important finding of the present study is the dissociation between the effects of stimulation of the ventral tegmental area on locomotor activity and prepulse inhibition. According to previous anatomical, electrophysiological and pharmacological studies, dopamine in the nucleus accumbens is important for the modulation of both locomotor activity and prepulse inhibition. Deficits in prepulse inhibition induced by dopamine agonists have been used as an animal model for neuropsychiatric disorders characterized by dysregulation of mesotelencephalic dopamine systems (Swerdlow et al., 1994). Our data clearly demonstrate that an enhancement of accumbal dopamine activity by stimulation of the ventral tegmental area enhanced locomotion, but did not reduce prepulse inhibition. Since prepulse inhibition deficits are induced by a challenging stimulation of the accumbal dopamine system (e.g. by the dopamine re-uptake inhibitor amphetamine), but not by a stimulus-driven synaptic release of dopamine as induced by stimulation of the ventral tegmental area, this clearly supports the idea that an experimentally induced prepulse inhibition deficit is a viable animal model for a pathological overactivation of the dopamine system, akin to the one underlying neuropsychiatric disorders like schizophrenia (Laruelle et al., 1996). The second important finding of the present study is that locomotor activity induced by a stimulation of the mesoaccumbal dopamine system is modulated by intranucleus accumbens adenosine A₁ receptors and dopamine D₁ receptors.

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References

- Borowski, T.B., Kokkinidis, L., 1996. Contribution of ventral tegmental area dopamine neurons to expression of conditioned fear: effects of electrical stimulation, excitotoxin lesions, and quinpirole infusion on potentiated startle in rats. *Behav. Neurosci.* 110, 1349–1364.
- Brockwell, N.T., Beninger, R.J., 1996. The differential role of A1 and A2 adenosine receptor subtypes in locomotor activity and place conditioning in rats. *Behav. Pharmacol.* 7, 373–383.
- Depoortere, R., Perrault, G., Sanger, D.J., 1997. Potentiation of prepulse inhibition of the startle reflex in rats: pharmacological evaluation of the procedure as a model for detecting antipsychotic activity. *Psychopharmacology* 132, 366–374.
- Dreher, J., Jackson, D.M., 1998. Role of D₁ and D₂ dopamine receptors in mediating locomotor activity elicited from the nucleus accumbens of rats. *Brain Res.* 487, 267–277.
- Ferré, S., 1997. Adenosine–dopamine interactions in the ventral striatum. Implications for the treatment of schizophrenia. *Psychopharmacology* 133, 107–120.
- Ferré, S., Popoli, P., Giménez-Llort, L., Finnman, U.B., Martínez, E., Scotti de Carolis, A., Fuxe, K., 1994. Postsynaptic antagonistic interaction between adenosine A1 and dopamine D₁ receptors. *NeuroReport* 6, 73–76.
- Gerlowitz, D.L., Kokkinidis, L., 1999. Enhanced amygdala kindling after electrical stimulation of the ventral tegmental area: implications of fear and anxiety. *J. Neurosci.* 19, 1–5.
- Ginés, S., Hillion, J., Torvinen, M., Le Crom, S., Casado, V., Canela, E.L., Randin, S., Lew, J.Y., Watson, S., Zoli, M., Agnati, L.F., Verniera, P., Lluis, C., Ferré, S., Fuxe, K., Franco, R., 2000. Dopamine D₁ and adenosine A1 receptors for functionally interacting heteromeric complexes. *Proc. Natl. Acad. Sci. U. S. A.* 97, 8606–8611.
- Hauber, W., Koch, M., 1997. Adenosine A_{2a} receptors in the nucleus accumbens modulate prepulse inhibition of the startle response. *NeuroReport* 8, 1515–1518.
- Hoffman, D.C., Donovan, H., 1994. D₁ and D₂ dopamine receptor antagonists reverse prepulse inhibition deficits in an animal model of schizophrenia. *Psychopharmacology* 115, 447–453.
- Hoffman, H.S., Ison, J.R., 1980. Reflex modification in the domain of startle: I. Some empirical findings and their implications for how the nervous system processes sensory input. *Psychol. Rev.* 87, 175–189.
- Kelley, A.E., Gauthier, A.M., Lang, G.C., 1989. Amphetamine microinjections into distinct striatal subregions cause dissociable effects on motor and ingestive behavior. *Behav. Brain Res.* 35, 27–39.
- Koch, M., Hauber, W., 1998. Regulation of sensorimotor gating by interactions of dopamine and adenosine in the rat. *Behav. Pharmacol.* 9, 23–29.
- Koch, M., Schmid, A., Schnitzler, H.-U., 2000. Role of nucleus accumbens dopamine D₁ and D₂ receptors in instrumental and Pavlovian paradigms of conditioned reward. *Psychopharmacology* 152, 67–73.
- Martin-Iverson, M.T., Else, D., 2000. PHNO, a selective dopamine D₂ receptor agonist, does not reduce prepulse inhibition of the startle reflex in rats. *Psychopharmacology* 151, 38–48.
- Meyer, M.E., 1993. Effects of intranucleus accumbens dopamine agonist SK&F38393 and antagonist SCH23390 on locomotor activities in rats. *Pharmacol. Biochem. Behav.* 45, 843–847.
- Mogenson, G.J., Wu, M., Manchanda, S.K., 1979. Locomotor activity initiated by microinfusions of picrotoxin into the ventral tegmental area. *Brain Res.* 161, 311–319.
- Mogenson, G.J., Brudzynski, S.M., Wu, M., Yang, C.R., Yim, C.C.Y., 1993. From motivation to action: a review of dopaminergic regulation of limbic-nucleus accumbens-ventral pallidum-pedunculopontine nucleus circuitries involved in limbic–motor integration. In: Kalivas, C.D., Barnes, C.D. (Eds.), *Limbic Motor Circuits and Neuropsychiatry*. CRC Press, Boca Raton, pp. 193–236.
- Laruelle, M., Abi-Dopaminergam, A., van Dyck, D.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., Carney, 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. U. S. A.* 93, 9235–9240.
- Ott, D.A., Mandel, R.J., 1995. Amphetamine sensitivity in open-field activity vs. the prepulse inhibition paradigm. *Brain Res. Bull.* 37, 219–222.
- Paxinos, G., Watson, C., 1997. *The Rat Brain in Stereotaxic Coordinates*. Academic Press, San Diego.
- Pellegrino, L.J., Pellegrino, A.S., Cushman, A.J., 1979. *A Stereotaxic Atlas of the Rat Brain Plenum*, New York.
- Popoli, P., Pézola, A., Scotti de Carolis, A., 1994. Modulation of striatal

- adenosine A1 and A2 receptors induces rotational behaviour in response to dopaminergic stimulation in intact rats. *Eur. J. Pharmacol.* 257, 21–25.
- Popoli, P., Giménez-Llort, L., Pezzola, A., Reggio, R., Martínez, E., Fuxe, K., Ferré, S., 1996. Adenosine A₁ receptor blockade selectively potentiates the motor effects induced by dopamine D₁ receptor stimulation in rodents. *Neurosci. Lett.* 218, 209–213.
- Ralevic, V., Burnstock, G., 1998. Receptors for purines and pyrimidines. *Pharmacol. Rev.* 50, 413–492.
- Schwarzkopf, S.B., Bruno, J.P., Mitra, T., 1993. Effects of haloperidol and SCH 23390 on acoustic startle and prepulse inhibition under basal and stimulated conditions. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 17, 1023–1036.
- Sills, T.L., Azampanah, A., Fletcher, P.J., 1999. The adenosine A₁ receptor agonist N⁶-cyclopentyladenosine blocks the disruptive effect of phencyclidine on prepulse inhibition of the acoustic startle response in the rat. *Eur. J. Pharmacol.* 369, 325–329.
- Swanson, C.J., Heath, S., Stratford, T.R., Kelley, A.E., 1997. Differential behavioral responses to dopaminergic stimulation of nucleus accumbens subregions in the rat. *Pharmacol. Biochem. Behav.* 58, 933–945.
- 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* 108, 189–195.
- Swerdlow, N.R., Braff, D.L., Caine, S.B., Geyer, M.A., 1993. Limbic cortico-striato-pallido-pontine substrates of sensorimotor gating in animal models and psychiatric disorders. In: Kalivas, P.W., Barnes, C.D. (Eds.), *Limbic Motor Circuits and Neuropsychiatry*. CRC Press, Boca Raton, pp. 311–328.
- Swerdlow, N.R., Braff, D.L., Taaid, N., Geyer, M.A., 1994. Assessing the validity of an animal model of deficient sensorimotor gating in schizophrenic patients. *Arch. Gen. Psychiatry* 51, 139–154.
- Swerdlow, N.R., Platten, A., Shoemaker, J., Pitcher, L., Auerbach, P., 2001. Effects of pergolide on sensorimotor gating of the startle reflex in rats. *Psychopharmacology* 158, 230–240.
- Turgeon, S.M., Pollack, A.E., Schusheim, L., Fink, J.S., 1996. Effects of selective adenosine A1 and A2A agonists on amphetamine-induced locomotion and *c-fos* in striatum and nucleus accumbens. *Brain Res.* 707, 75–80.
- Zhang, J., Engel, J.A., Hjorth, S., Svensson, L., 1995. Changes in the acoustic startle response and prepulse inhibition of acoustic startle in rats after local injection of pertussis toxin into the ventral tegmental area. *Psychopharmacology* 119, 71–78.
- Zhang, J., Forkstam, C., Engel, J.A., Svensson, L., 2000. Role of dopamine in prepulse inhibition of acoustic startle. *Psychopharmacology* 149, 181–188.