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Inhibition of NMDA-induced striatal dopamine release and behavioral activation by the neuroactive steroid 3α-hydroxy-5β-pregnan-20-one hemisuccinate

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Abstract

Our laboratory has previously shown that the synthetic neuroactive steroid 3α-hydroxy-5β-pregnan-20-one hemisuccinate (3α5βHS) is a negative modulator of NMDA receptors *in vitro*. Similarly, 3α5βHS exhibits rapid sedative, analgesic, anticonvulsive, and neuroprotective effects *in vivo*. Here we report a study designed to investigate whether a negatively charged neuroactive steroid, 3α5βHS, modulates the action of NMDA receptors *in vivo*. Our results indicate that peripherally administered 3α5βHS enters the CNS and inhibits NMDA-mediated motor activity and dopamine release in the rat striatum. The increase in motor activity induced by intrastriatal microinjection of NMDA was blocked by the systemic administration of 3α5βHS and the NMDA-induced increase in extracellular dopamine in the striatum was also attenuated by both systemically administered and intrastriatally administered (by *in vivo* microdialysis) 3α5βHS. These data indicate that 3α5βHS acts through striatal NMDA receptors *in vivo*. When taken together, these results suggest that neuroactive steroids may prove to be effective in the treatment of neurological and psychiatric disorders involving over-stimulation of NMDA receptors in the mesotelencephalic dopamine system.

Keywords: dopamine, microdialysis, modulator, NMDA receptor, neuroactive steroid, striatum.

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Neuroactive steroids have been proposed to play a role in the modulation of inhibitory and excitatory amino acid receptor function. Considerable evidence suggests that endogenous neurosteroids, such as allopregnanolone, may act as physiological modulators of $GABA_A$ receptor function through a non-genomic mechanism *in vivo* and *in vitro* (Paul and Purdy 1992; Rabow *et al*. 1995; Baulieu 1998). Pregnenolone sulfate, which is a negatively charged sulfated steroid, is found in the CNS and has been shown to modulate the actions of glutamate at ionotropic amino acid receptors in neurons and in transient expression systems (Wu *et al*. 1991; Weaver *et al*. 1997; Yaghoubi *et al*. 1998). In particular, pregnenolone sulfate potentiates NMDA receptor-mediated activity (Wu *et al*. 1991; Irwin *et al*. 1992; Bowlby 1993; Wong and Moss 1994) of NR2A- and NR2B-containing receptors while inhibiting NR2C- and NR2D-containing receptors (Malayev *et al*. 2002). Pregnenolone sulfate increases the potency and efficacy of NMDA in multiple assays, including NMDA-induced currents in primary neurons and *Xenopus* occytes expressing NR1 and NR2A receptor subunits (Wu *et al*. 1991; Yaghoubi *et al*. 1998), NMDA-induced increases in intracellular calcium (Irwin *et al*. 1992), and NMDA-induced excitotoxicity of hippocampal neurons in culture (Weaver *et al*. 1998).

Conversely, pregnanolone sulfate (3α-ol-5β-pregnan-20-one sulfate), the sulfated form of the neurosteroid pregnanolone, decreases NMDA-induced increases in current (Park-Chung *et al*. 1994) and NMDH-induced increases in the

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Abbreviations used: 3α5βHS, 3α-hydroxy-5β-pregnan-20-one hemisuccinate; aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; D-AP5, D-(–)-2-amino-5-phosphonopentanoic acid; HPLC, high-performance liquid chromatography; PAHG, 3α-hydroxy-5β-pregnan-20-one hemiglutarate; PEHS, pregnenolone hemisuccinate.

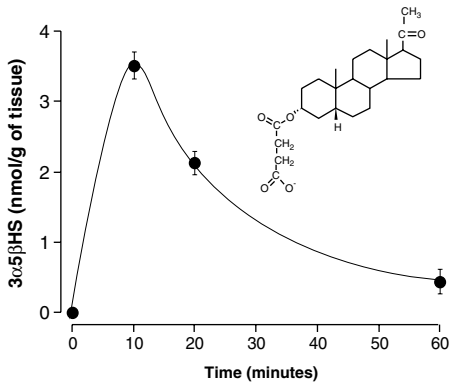

Fig. 1 3α5βHS rapidly enters the brain following systemic injection. Male rats were administered 3α5βHS (10 mg/kg, i.p.). 3α5βHS was extracted from whole brain, and analyzed via HPLC ESI-MS. Data represent the mean nmol/g 3α5βHS in whole brain obtained from three animals ± SEM. Note that 3α5βHS enters the brain within 10 min after injection and declines to near baseline levels by 60 min. Data represent the average nmol/g 3α5βHS of three animals ± SEM. Inset: Chemical structure of 3α5βHS.

intracellular calcium (Irwin *et al*. 1994) of neurons in culture. The enhancement of NMDA receptor activity by pregnenolone sulfate and the inhibition by pregnanolone sulfate occur through independent sites located on the extracellular surface of NMDA receptors (Park-Chung *et al*. 1997).

The activity of endogenous neuroactive steroids at NMDA receptors is dependent upon the presence of a sulfate group at the C3 position. Structure–activity studies with isolated neurons and *Xenopus* oocytes expressing NMDA receptor subunits demonstrate that the principal requirement is for a negative charge in the C3 position (Park-Chung *et al*. 1994). For example, the sulfate group may be replaced by a hemisuccinate while maintaining substantial activity: 3α-ol-5β-pregnan-20-one hemisuccinate [pregnanolone hemisuccinate (3α5βHS); Fig. 1a] has similar *in vitro* activity to pregnanolone sulfate. Steroids bearing a hemisuccinate group may be more stable, due to their resistance to endogenous sulfatases. Moreover, the hemisuccinated steroids have a substantially higher pK_a than the sulfated form and will be partly unionized at physiological pH, which allows the hemisuccinated steroids to penetrate the blood–brain barrier more readily (Weaver *et al*. 1997).

In a previous study, 3α5βHS was shown to inhibit NMDA-induced cell death in rat hippocampal neurons in culture (Weaver *et al*. 1997). 3α5βHS (25 mg/kg) produced sedation in mice and was antinociceptive in late phase formalin-induced pain after peripheral administration. Furthermore, intravenous injections of 3α5βHS significantly reduced cortical and subcortical infarct size following occlusion of the middle cerebral artery. These findings are consistent with distinct negative modulation of NMDA receptors by 3α5βHS (Weaver *et al*. 1997). The negative modulation of NMDA receptors by 3α5βHS is of particular interest in the context of therapeutics, given the role of NMDA receptors in a constellation of neurological and psychiatric disorders including drug craving, epilepsy, hypoxic neuronal damage, schizophrenia, excitotoxicity, and Parkinson's disease.

In the present study we determined the time course of 3α5βHS appearance in the CNS by measuring steroid levels in the brain following systemic administrations. Based on this time course, we determined the effect of 3α5βHS [intraperitoneal (i.p.)] on locomotor activity induced by microinjection of NMDA into the rat striatum, and on dopamine release induced by perfusion of NMDA via *in vivo* microdialysis.

Materials and methods

Subjects

Male Sprague–Dawley rats (225–300 g) from Charles River Laboratories (Wilmington, MA, USA) were initially housed in shoebox cages (two rats/cage) and were provided with food and water *ad libitum*. The cages were kept in a temperature-controlled room with a 12-h light/dark cycle. All experiments were performed during the light cycle. This research was carried out under a protocol approved by the Boston Universty School of Medicine IACUC.

Materials

All steroids were purchased from Steraloids Inc. (Wilton, NH, USA) and were dissolved in dimethyl sulfoxide (DMSO; 100%) and injected systemically at 0.5 mL/kg. The amount of DMSO used in our studies never exceeded 150 μL/animal. It has previously been shown that DMSO at concentrations of 5–90% does not disrupt the blood–brain barrier (Neuwelt *et al*. 1983; Walters *et al*. 1984; Greig *et al*. 1985; Authier *et al*. 2002). NMDA and D-(–)-2-amino-5-phosphonopentanoic acid (D-AP5) were purchased from Sigma (St Louis, MO, USA) and dissolved in artificial cerebrospinal fluid (aCSF; 145 mM NaCl; 2.7 mM KCl; 1.2 mM $CaCl_2$; 1.0 mM $MgCl_2$; 0.2 mM ascorbate; 5.0 mM glucose; pH 7.4).

Determination of pregnanolone hemisuccinate tissue levels

Rats were housed for one week prior to initiation of experiments. On the day of the experiment, rats were divided into three groups based on their scheduled time of death (10, 20 or 60 min post-injection) and administered 3α5βHS (10 mg/kg, i.p.). Rats were anesthetized with isoflurane, and decapitated at the designated time points. Brain tissue was rapidly removed, dissected, and stored at − 80°C until assayed.

For each rat 0.6–2 g of tissue homogenate from whole brain was analyzed for 3α5βHS; the larger tissue amounts were required for samples originating from the 60 min time-point. Pregnanolone hemiglutarate (PAHG) 1 μL of a 2.7-mM solution in ethanol was added to tissue samples for use as an extraction recovery standard. Tissues were homogenized in 2 mL of 0.25 M NaOH with a sonic dismembrator. Potentially interfering lipids were extracted from the alkaline homogenates with 15 mL of n-hexane in three aliquots. Homogenates were then acidified by the addition of 2.5 mL of 2.5 M H_2PO_4. 3α5βHS and PAHG were then extracted from the

homogenates with the aid of a sonic dismembrator using three 5 mL aliquots of 10% ethyl acetate in hexane.

Extraction aliquots were combined, diluted with 10 mL of n-heptane, and loaded onto a conditioned 500 mg silica SepPak® column (Waters Corp., Milford, MA, USA). Columns were washed with 5 mL hexane, and steroidal analytes were eluted with 3 mL of a 1 : 1 mixture of acetone : hexane. The eluant was evaporated to dryness under vacuum and reconstituted in 49 μL of CH_3OH and 1 μL of 50 mM ammonium acetate containing 2.5 pmol pregnenolone hemisuccinate (PEHS) for use as an instrument standard.

Chromatographic resolution was achieved using a HP 1100 high-performance liquid chromatography (HPLC) running a mobile phase of 75% CH_3OH, 25% 50 mM ammonium acetate (pH 3) at a flow rate of 100 μL/min through a Hypersil BDS C18 column (2.1 × 150 mm; Alltech Associates, Deerfield, IL, USA). Column effluent was directed into a HP 5989x electrospray mass spectrometer operating in the negative ion mode and running a sheath liquid of 50 mM NH_4OH at 5 μL/min. The mass spectrometer was set to monitor the deprotonated ions of the analyte and two standards (3α5βHS m/z = 417.2, PAHG m/z = 431.3, PEHS m/z = 415.2).

Sample values of 3α5βHS were normalized to the optimal response of the instrument standard, and then determined by comparing to a concurrently run, external standard curve. 3α5βHS values were then corrected for extraction efficiency, which was determined by monitoring PAHG recovery.

Microinjection experiments

Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and placed in a stereotaxic apparatus (David Kopf Instruments, Tujung, CA, USA). A rostro-caudal incision was then made to expose the dorsal surface of the skull. Two bilateral holes were drilled above the striatum (+ 1.0 mm A/P, + 3.0 mm M/L, − 3.0 mm D/V relative to bregma, Paxinos and Watson 1997) and guide cannulae (9 mm, 24 gauge) were implanted and fastened to the skull using screws and dental cement. All rats were housed in individual cages following surgery.

Three days post surgery, rats were habituated to the photocell apparatus (AccuScan Instruments; Columbus, OH, USA) for 2 h. On the day of the experiment rats were rehabituated for 1 h prior to the start of the experiment. Vehicle or 3α5βHS (1 or 10 mg/kg, i.p.) was administered systemically prior to microinjection. Ten minutes following the injection, obturators were removed from the guide cannulae and replaced by a 33-gauge stainless steel injection needle that extended 2 mm below the tip of the guide cannulae into the striatum. Bilateral infusions of NMDA (10 μg/μL) or 0.9% saline were made over 2 min at a volume of 0.5 μL per side into the striatum. The injectors remained in the striatum for 1 min following the injection to ensure that the compounds had diffused from the site of injection. The animals were returned to the behavior boxes and activity was monitored continuously for 60 min.

Another group of rats were administered with either vehicle or one of the following doses of 3α5βHS (1, 5, 10, or 20 mg/kg, i.p.). Ten minutes after the injection, the animals were placed in photocell boxes and motor activity was measured for 1 h.

In vivo microdialysis

Prior to surgery the animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and placed in a stereotaxic apparatus (David Kopf Instruments). A rostro-caudal incision was then made to expose the dorsal surface of the skull. A hole was drilled in the skull above the striatum with a stereotaxic drill (+ 0.5 mm A/P and + 2.9 mm M/L relative to bregma; Paxinos and Watson 1997). Two other holes were drilled in the skull where screws were secured. A dialysis guide cannula (CMA Microdialysis, Acton, MA, USA) was lowered 2 mm ventrally and was then secured to the skull with dental cement. All rats were housed in individual cages following surgery.

No fewer than 2 days after surgery, the rats were briefly anesthetized with isoflurane to facilitate the removal of the dummy from the guide cannula and insertion of a microdialysis probe (CMA 10, dialysis membrane length of 2 mm of polycarbonate, with a molecular weight cut-off of 20 kDa) into the striatum. Animals were allowed to recover for 120 min prior to the initiation of the experiment following probe insertion while aCSF was pumped through the dialysis probe at a rate of 2 μL/min. Twelve 20-min samples were collected over 240 min and immediately stored at −20°C. The first six 20-min samples were collected to establish the baseline concentration of dopamine. Following samples 1–6, the rats were administered either vehicle or 3α5βHS (0.01, 1, 5, 10 or 20 mg/kg, i.p.) before the collection of sample 7. At this point the perfusate for the vehicle group was changed from aCSF to one of the following solutions: NMDA (1 mM) or NMDA (1 mM) plus D-AP5 (100–200 μM). The perfusate for the steroid group was switched from aCSF to NMDA (1 mM). After collection of sample 7, the perfusate was switched back to aCSF and five more 20-min samples were collected. Upon termination of the experiment, samples were stored at −80°C until analysis.

A second group of rats was briefly anesthetized with isoflurane and the microdialysis probe was inserted into the striatum no less than 12 h prior to the start of the experiment. Artificial cerebrospinal fluid was pumped at a rate of 0.2 μL/min during the 12 h. Preceding the start of the experiment the flow rate was gradually increased to 2 μL/min and the dialysis experiment was begun following the same protocol as described above.

A third group of rats was also briefly anesthetized with isoflurane and the microdialysis probe was inserted into the striatum no less than 12 h prior to the start of the experiment. Artificial cerebrospinal fluid was pumped at a rate of 0.2 μL/min during the 12 h. Preceding the start of the experiment the flow rate was gradually increased to 2 μL/min. The first six 20-min samples were collected to establish the baseline concentration of dopamine. Following samples 1–6, the perfusate was changed from aCSF to one of the following solutions: NMDA (1 mM) + vehicle or NMDA + 3α5βHS (100 μM) and sample 7 was collected. After collection of sample 7, the perfusate was switched back to aCSF and five more 20-min samples were collected. Upon termination of the experiment, samples were stored at −80°C until analysis.

All samples were analyzed using HPLC with electrochemical detection using a carbon detector (ESA, Chelmsford, MA, USA). The mobile phase consisted of 50 mM Na_2HPO_4, 20 mM citric acid, 1.5 mM heptanosulfonic acid, 0.1 mM EDTA, and 5% methanol at pH 4.0. The dopamine was separated on a 15-cm reverse phase C-18 column (Alltech) and oxidized at a potential of 175 mV. Dopamine concentrations per sample were normalized to an external standard curve and plotted as percentage change from baseline.

Histology

Upon completion of the microinjection and microdialysis experiments the animals were given an overdose of sodium pentobarbital (100 mg/kg, i.p.) and were perfused intracardially with 0.9% saline followed by 10% formalin. The brains were removed and coronal sections (100 μm) were taken at the level of the striatum using a Vibratome (Technical Products International, St Louis, MO, USA). The sections were stained with Cresyl violet. Cannulae placement and potential neurotoxicity were determined by an individual who was unaware of prior treatments and images were recorded using a Nikon Eclipse 6500 microscope with a Spot insight color camera and software (Diagnostic Instruments Inc., Sterling Heights, MI, USA).

Results

Time course of 3α5βHS appearance in brain

To assess the rate of appearance and disappearance of steroid in the brain, rats were administered with 3α5βHS (10 mg/kg, i.p.) and killed at 10, 20, and 60 min post injection. 3α5βHS was extracted from whole brain, and analyzed via HPLC ESI-MS. 3α5βHS was detected in the brain within 10 min (Fig. 1). Steroid levels reached 3.5 nmol/g of brain tissue at 10 min post injection and declined to 0.5 nmol/g of brain tissue by 60 min. 3α5βHS levels peak within 10 min post injection and the concentration in whole brain remains high for at least 20 min post injection.

Histological examination

Figure 2 represents the histological analysis of brain tissue following microdialysis and microinjection experiments. The brain sections were examined under a microscope following Nissl staining. From the cumulative visual inspection of approximately one hundred such experiments, some gliosis was detected immediately surrounding the probe or microinjection site. As shown in Figs 2(a and b), in representative sections from striatum of a microdialysis experiment, there was no indication of NMDA-induced toxicity as the characteristics of the surrounding tissue as judged by the extent of glial proliferation and presence of healthy looking cells did not differ between aCSF and NMDA treatments. Some expected mechanical damage was detected secondary to probe placement and cannulation. Histological results also indicate placement of the dialysis probes and the microinjection cannulae within the dorsal striatum (Figs 2c and d).

Effect of 3α5βHS on baseline motor activity

As shown in Table 1, motor activity counts were not significantly affected following 3α5βHS administration (1, 5, 10, or 20 mg/kg). These results indicate that 3α5βHS does not decrease baseline motor activity and therefore it is not sedative at any of the doses tested (Table 1). Although there was no significant difference between the 20 mg/kg dose and control groups, we reduced the concentration to 10 mg/kg in

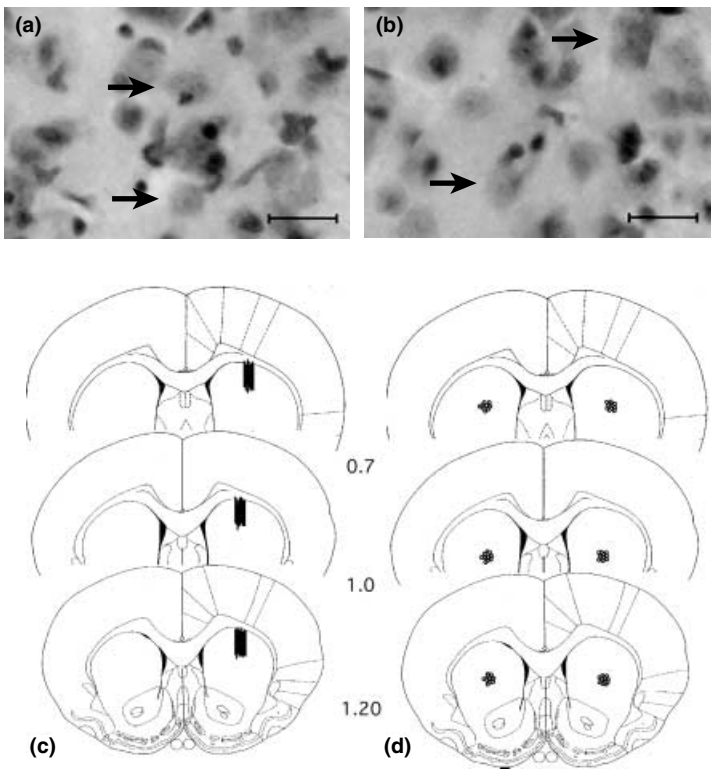

Fig. 2 Histological analysis and location of cannulae. The cannula tracks are located at the superior border of the image. (a) Bright field photomicrograph of a sample saline-treated rat. (b) Bright field photomicrograph of a sample NMDA-treated rat. Note the similar number of neurons (arrows) and glia in the histologies from saline- and NMDA-treated rats. (c) Location of microdialysis probe placement. (d) Location of microinjection guide cannulae. Scale bar 20 μm.

Table 1 The effect of 3α5βHS on baseline motor activity

	Activity counts ± SEM
Vehicle	8130 ± 2162
3α5βHS (1 mg/kg)	8611 ± 1211
3α5βHS (5 mg/kg)	8038 ± 1383
3α5βHS (10 mg/kg)	8453 ± 2741
3α5βHS (20 mg/kg)	4361 ± 1891

Rats were administered vehicle or 3α5βHS (1, 5, 10, or 20 mg/kg) and placed in behavioral boxes and motor activity was measured for 1 h. No dose of 3α5βHS tested produced a significant change in baseline locomotor activity. There were four animals per group.

our behavioral studies in order to further ensure that there would be no confounding direct inhibition of motor activity.

3α5βHS blocks the behavioral hyperactivity induced by intrastriatal NMDA

Rats were administered vehicle or one of two doses of 3α5βHS (1 mg/kg or 10 mg/kg) intraperitoneally 10 min prior to intrastriatal microinjection of NMDA or saline. Horizontal activity was measured in 5-min blocks for 60 min immediately following microinjection. The data in Fig. 3(a) represent the 10-min time point from the time course (Fig. 3b) and was analyzed using a one-way analysis of variance (ANOVA). While this analysis revealed a marginally significant main effect ($F_{4,32} = 2.577$, $p < 0.0562$), post-hoc (Fisher's LSD) analyses indicated that the NMDA-induced behavioral activation was blocked by 3α5βHS (1 and 10 mg/kg; Fig. 3a). The data in Fig. 3(b) were analyzed with a mixed factors ANOVA with repeated measures over time. The analysis revealed a significant main effect of time ($F_{11,352} = 6.63$, $p < 0.0001$) and a marginally significant main effect of treatment ($F_{44,32} = 2.54$, $p < 0.058$). There was no significant treatment–time interaction. 3α5βHS (10 mg/kg) did not induce sedation, reflected by no significant difference between the saline + vehicle and the saline + 3α5βHS groups at any time point (Fig. 3).

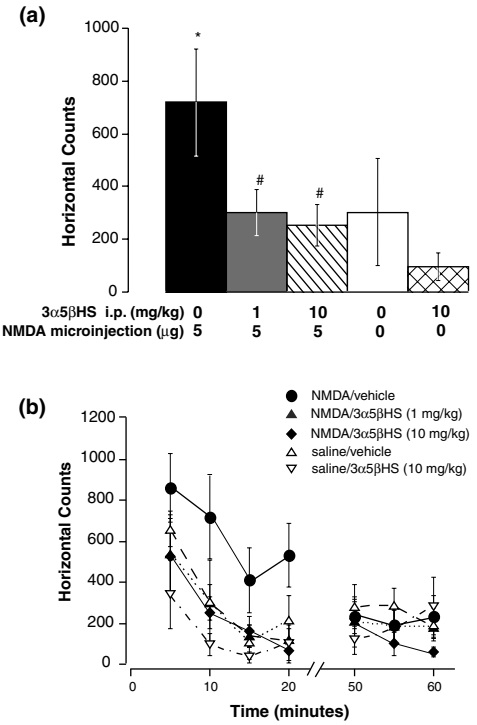

Fig. 3 3α5βHS blocks behavioral activity induced by NMDA in the rat striatum. (a) 3α5βHS (1 or 10 mg/kg) or vehicle were administered i.p. 10 min prior to the microinjection of NMDA (10 μg/mL) or sterile saline into the rat striatum. Following microinjection, animals were placed in behavioral boxes and horizontal activity was monitored at 5-min intervals for 1 h. Bar graph represents the horizontal count at 10 min post-NMDA microinjection. *Denotes significant increase in horizontal activity compared to the saline group, while # represents significant difference between treatment groups as compared to control ($p < 0.05$, Fisher's LSD). The animals treated with NMDA/vehicle show an increase in horizontal activity as compared to the both saline/vehicle and saline/3α5βHS groups. However, animals treated with NMDA/3α5βHS (1 or 10 mg/kg) show a decrease in horizontal activity compared to the NMDA/vehicle group. (b) Time-course of 3α5βHS inhibition of NMDA-induced behavioral activity. Number of animals used in microinjection experiments was six to eight per group. ●, NMDA/vehicle; ▲, NMDA/3α5βHS (1 mg/kg); ◆, NMDA/3α5βHS (10 mg/kg); △, saline/vehicle; ▽, saline/3α5βHS (10 mg/kg).

3α5βHS attenuates NMDA-induced dopamine release

Basal extracellular levels of striatal dopamine were measured in 52 rats via *in vivo* microdialysis. The average (± standard error of the mean) extracellular dopamine value was 2.0 ± 0.8 nM/20 μL.

Systemic administration of 3α5βHS (0.01, 1, 5, 10 or 20 mg/kg) decreased NMDA-induced dopamine release dose-dependently (Fig. 4). The data are presented as percentage baseline, with baseline defined as the average of the four samples collected prior to administration of NMDA. The complete time-course of the microdialysis data is presented in Fig. 4(a). The complete time-course was analyzed with a mixed factors ANOVA (repeated measures over time). The results of this analysis revealed significant main effects of treatment ($F_{5,22} = 5.52$; $p < 0.0019$) and time ($F_{8,176} = 37.5$, $p < 0.0001$) as well as a significant treatment–time interaction ($F_{40,176} = 4.16$, $p < 0.0001$). The data collected 20 and 40 min following the intrastriatal perfusion of NMDA is presented in Fig. 4(b). The data in Fig. 4(b) were analyzed with a one-way ANOVA and the analysis revealed significant main effects at 20 min ($F_{5,22} = 4.455$, $p < 0.0059$) and 40 min ($F_{5,22} = 5.16$, $p < 0.0028$). Subsequent pairwise analyses (Fisher's LSD) showed that there was a significant increase in extracellular dopamine in the striatum 20 and 40 min following perfusion of NMDA, and that this effect was blocked by 3α5βHS at the following doses, 5, 10, and 20 mg/kg.

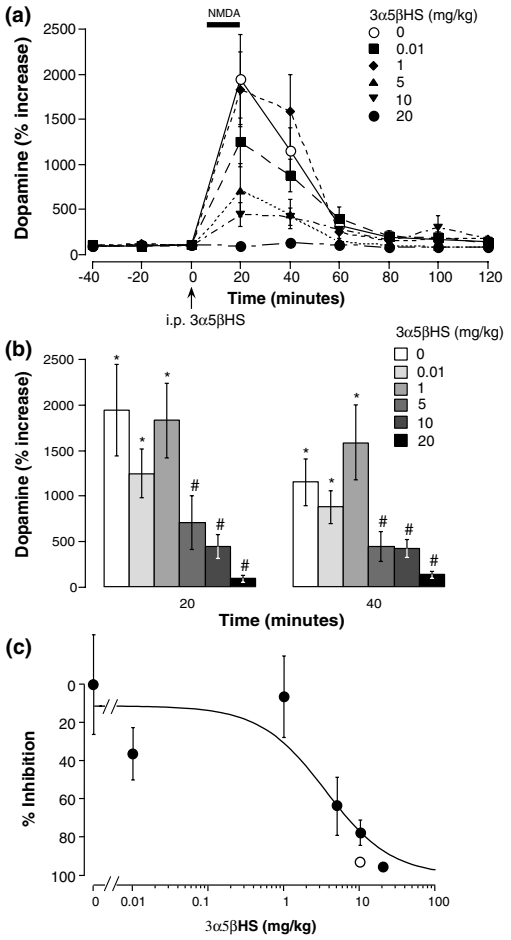

Fig. 4 3α5βHS attenuates NMDA-evoked release of extracellular dopamine in the rat striatum. (a) Rats were injected with either vehicle or 3α5βHS (○, 0; ■, 0.01; ◆, 1; ▲, 5; ▼, 10; or ●, 20 mg/kg, i.p.) immediately after collection of the sample at time 0. After a 10-min waiting period, the perfusion medium was switched from aCSF to a 1-mM NMDA solution dissolved in aCSF. After collection of the next 20 min sample, the perfusion medium was switched back to aCSF and five more samples were collected. Data are mean ± SEM values for extracellular concentrations of dopamine, presented as percentages of baseline values. The infusion of 1 mM NMDA solution started at the 10-min time point and lasted for 20 min (indicated by horizontal bar). (b) Data represents peak times for 20 and 40 min from (a). 3α5βHS inhibits NMDA-induced dopamine release at a 5, 10 or 20 mg/kg dose. *Denotes significant difference from baseline levels of dopamine for the 5, 10 and 20 mg/kg dose, while # represents significant difference between treatment groups compared to control ($p < 0.05$, Fisher's LSD). (c) Dose–response curve for 3α5βHS inhibition of NMDA-induced dopamine release at 20 min. ○, Data for group of rats that had microdialysis probe inserted 12 h before the start of the experiment. Data points: percentage change in NMDA-induced striatal dopamine release with an i.p. injection of 3α5βHS (mean of four to six experiments). Error bars: SEM. Curve is a non-linear least-squares fit to the Michealis–Menton equation; EC_{50} for 3α5βHS is 4 mg/kg. For the *in vivo* microdialysis experiments the number of animals used was four to six per group.

In order to ensure that a 120-min waiting period before the start of sample collection was a sufficient amount of time for dopamine levels to reach equilibrium following probe implantation, we repeated our dialysis experiments with the 10 mg/kg dose of 3α5βHS 12 h following insertion of the dialysis probe. Systemic administration of 3α5βHS (10 mg/kg) decreased NMDA-induced dopamine release in the group of rats that had the dialysis probe inserted 12 h prior to the start of the experiment (Fig. 4c, ○). The results of this analysis revealed significant main effects of treatment ($F_{1,7} = 5.43$; $p < 0.05$) and time ($F_{8,56} = 5.0$, $p < 0.0001$) as well as a significant treatment–time interaction ($F_{8,56} = 3.42$, $p < 0.0028$). The NMDA-induced increase in dopamine was dose-dependently decreased by 3α5βHS with an EC_{50} of 4 mg/kg (Fig. 4c).

The data presented in Fig. 5 indicate that vehicle administration has no effect on basal dopamine release, and that NMDA-induced striatal dopamine release is attenuated by the NMDA antagonist D-AP5. These results suggest that NMDA receptor activation is necessary for striatal dopamine release. The data are presented as percentage baseline, with baseline defined as the average of the four samples collected prior to administration of NMDA. The complete time-course

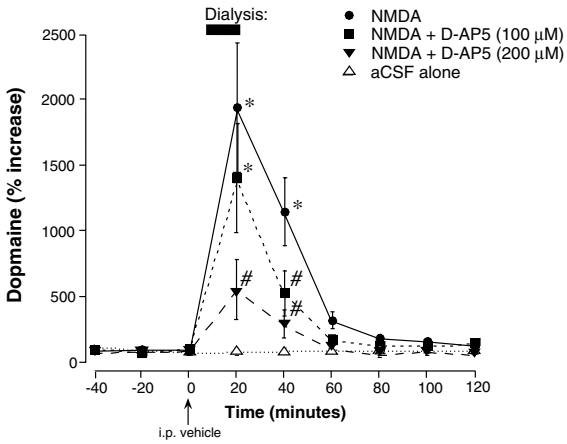

Fig. 5 D-AP5 inhibits NMDA-evoked release of extracellular dopamine in the rat striatum. Rats were injected with vehicle (●) immediately after collection of the sample at time 0. After a 10-min waiting period, one group of rats was perfused with aCSF alone (△), while in the other three groups the perfusion medium was switched from aCSF to a 1-mM NMDA solution with D-AP5 (■, 100 μM; ▼, 200 μM) dissolved together in aCSF. After collection of the next 20-min sample, the perfusion medium was switched back to aCSF and five more samples were collected. The infusion of NMDA (1 mM) + D-AP5 (100 μM or 200 μM) solution started at time 10 and lasted for 20 min (indicated by horizontal bar). Data are mean ± SEM for extracellular concentrations of dopamine, presented as percentages of baseline values. *Denotes significant difference from baseline levels of dopamine, while # represents significant difference between treatment groups compared to aCSF ($p < 0.05$, Fisher's LSD). For the *in vivo* microdialysis experiments the number of animals used was four to six per group.

of the microdialysis data was analyzed with a mixed factors ANOVA with repeated measures over time (Fig. 5a). The results of this analysis revealed significant main effects of treatment ($F_{2,12} = 3.83$; $p < 0.05$) and time ($F_{8,96} = 16.57$, $p < 0.0001$) as well as a significant treatment–time interaction ($F_{16,96} = 1.84$, $p < 0.036$). The data collected 20 and 40 min following the intrastriatal perfusion of NMDA are presented in Fig. 5(b). Data in Fig. 5(b) were analyzed with a one-way ANOVA, which revealed a significant main effect at 40 min [$F_{2,12} = 3.875$, $p < 0.0503$] that was not observed at 20 min. Subsequent pairwise analyses (Fisher's LSD) showed that there was a significant increase in extracellular dopamine in the striatum 20 and 40 min following perfusion of NMDA through the microdialysis probe. The NMDA-induced increase in dopamine was attenuated in a dose-dependent manner by the co-perfusion of 100 μM or 200 μM D-AP5 with NMDA (Fig. 5).

Similarly, 3α5βHS (100 μM) perfused together with NMDA (1 mM) through the dialysis probe inhibited striatal dopamine release (Fig. 6). The data are presented as percentage baseline, with baseline defined as the average of the four samples collected prior to administration of NMDA. The complete time course of the microdialysis data is presented in Fig. 6. Analysis by mixed factors ANOVA (repeated measures over time) revealed significant main effects of treatment ($F_{1,6} = 7.65$; $p < 0.0328$) and time ($F_{8,48} = 10.911$, $p < 0.0001$) as well as a significant treatment–time interaction ($F_{8,48} = 3.415$, $p < 0.0035$). Subsequent pairwise analyses (Fisher's LSD) showed that there was a significant increase in extracellular dopamine in the striatum at 20 min following perfusion of NMDA and that this effect was attenuated by 3α5βHS.

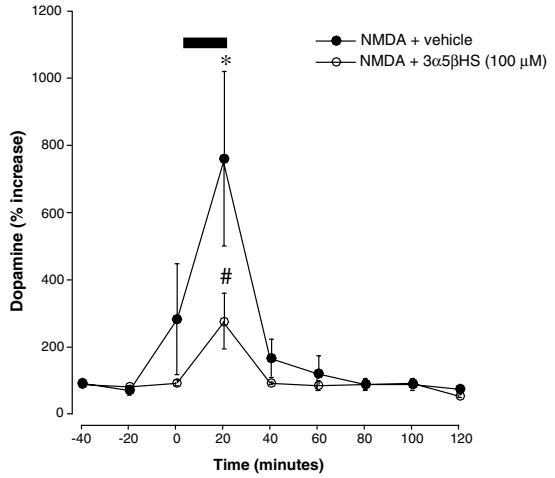

Fig. 6 3α5βHS perfused through the dialysis probe attenuates NMDA-evoked release of extracellular dopamine in the rat striatum. After the collection of sample at time 0, one group of rats was perfused with NMDA (1 mM) + vehicle (●) while the other group was perfused with NMDA (1 mM) + 3α5βHS (○, 100 μM) solution dissolved together in aCSF. After collection of the next 20-min sample, the perfusion medium was switched back to aCSF and five more samples were collected. Data are mean ± SEM for extracellular concentrations of dopamine, presented as percentages of baseline values. The infusion of NMDA solutions started at time 10 and lasted for 20 min (indicated by horizontal bar). For the *in vivo* microdialysis experiments the number of animals used was four to five per group.

Discussion

Evidence indicates that neuroactive steroids such as 3α5βHS inhibit NMDA receptor activation in primary cultures of neurons and in *Xenopus* oocytes expressing cloned human NMDA receptors (Weaver *et al*. 1997; Yaghoubi *et al*. 1998). However, the question of whether 3α5βHS and related neurosteroids act via the NMDA receptor *in vivo* remains unanswered. Our present results demonstrating that 3α5βHS inhibits NMDA-induced increases in motor activity combined with the finding that 3α5βHS blocks NMDA-induced dopamine release in the rat striatum, strongly suggest that 3α5βHS negatively modulates NMDA receptors *in vivo*.

Time-course of 3α5βHS action

The pharmacokinetic results indicate that 3α5βHS is at its highest concentration in the brain approximately 10 min post-administration. Experiments were designed such that NMDA microinjection or NMDA infusion through the dialysis probe was initiated 10 min following i.p. steroid administration. The pharmacokinetic results were also used to determine the duration of sample collection for the microdialysis experiments. The first 20-min collection period was initiated 10 min post-administration of 3α5βHS in order to capture the effect of the peak brain concentration of 3α5βHS on NMDA-induced dopamine release.

3α5βHS blocks locomotor hyperactivity induced by intrastriatal microinjection of NMDA

The ability of 3α5βHS to dose-dependently decrease the behavioral response elicited by intrastriatal microinjections of NMDA is consistent with *in vitro* studies showing that 3α5βHS inhibits NMDA receptors (Park-Chung *et al*. 1994) and studies showing that excitatory amino acids regulate striatal-mediated motor activity in that NMDA infused into the nucleus accumbens resulted in an increase in behavioral activation (Riederer *et al*. 1992; Schmidt *et al*. 1992; Ossowska and Wolfarth 1995). The attenuation of NMDA-induced behavioral hyperactivity by 3α5βHS does not appear to be due to a sedative effect of the steroid since the highest dose used had no effect on the non-pharmacological behavioral activation induced by saline administration or on baseline motor activity following systemic administration. Furthermore, this same dose of 3α5βHS had no effect on the behavioral hyperactivity induced by an acute cocaine injection (data not shown).

Administration of NMDA at concentrations of 1 mM or higher into the striatum increases extracellular levels of

dopamine (Cheramy *et al*. 1986; Moghaddam *et al*. 1990; Keefe *et al*. 1992), and the concentration of NMDA microinjected in our behavioral experiment (0.1 μg/0.5 μL) was slightly higher than 1 mM. Because increases in extracellular dopamine in the nucleus accumbens are well known to increase motor activity (Wu *et al*. 1993; Taepavarapruk *et al*. 2000), we hypothesize that the increase in motor activity seen in rats receiving striatal microinjections of NMDA is secondary to increased NMDA-induced dopamine release and that 3α5βHS inhibits this motor activity via negative modulation of the NMDA receptor.

While 3α5βHS (1 mg/kg, i.p.) had no significant effect on dopamine release induced by intrastriatal administration of NMDA via microdialysis (Fig. 4), this same dose of 3α5βHS inhibits behavioral hyperactivity induced by intrastriatal microinjection of NMDA. The lack of exact correlation between these two effects at a single low dose of 3α5βHS could be explained by several possible mechanisms. Whereas behavioral experiments measure changes in the total effect of a multineuronal circuit distal to the site of NMDA microinjection, microdialysis experiments measure changes in dopamine release at the site of NMDA administration. Microinjection of NMDA stimulates local striatal neurons leading to a chemical cascade that propagates across the striatum and other basal ganglia nuclei in order to initiate motor behavior via glutamatergic projections at distant synapses. The dose–effect analysis demonstrates an ED_{50} of 4 mg/kg (Fig. 4c), consistent with approximately 20% inhibition of NMDA-induced dopamine release at 1 mg/kg 3α5βHS. Given that 3α5βHS (i.p.) is likely to be present throughout the brain, possibly affecting all involved synapses, it may be that the cumulative effect of this level of inhibition is sufficient to reduce motor activation. Alternatively, 3α5βHS may be more potent at glutamatergic synapses that are involved in NMDA-induced behavioral hyperactivity than at those that directly regulate dopamine release. While the subunit dependence of 3α5βHS potency has not been determined, the potency of pregnanolone sulfate differs by about fourfold among NMDA receptors, depending on subunit composition (Malayev *et al*. 2002).

3α5βHS attenuates NMDA-induced dopamine release in the striatum

NMDA receptor mRNA has been localized to both GABAergic and cholinergic interneurons as well as GABAergic projection neurons in the striatum (Landwehrmeyer *et al*. 1995; Kuppenbender *et al*. 2000). In addition, anatomical studies indicate that glutamate receptors are present on striatal dopaminergic nerve terminals (Sesack and Pickel 1990). Given the diverse localization of NMDA receptors in the striatum it is difficult to distinguish whether synaptically released glutamate acts at pre-, post-, or pre- and post-synaptic glutamatergic sites to modulate striatal dopamine release. In the striatum, evidence for pre-synaptic control of dopamine release by glutamate is derived from the lack of inhibition of glutamate-induced release of dopamine by tetrodotoxin using *in vivo* microdialysis (Westerink *et al*. 1989; Keefe *et al*. 1992; Martinez-Fong *et al*. 1992), and push–pull cannulation (Cheramy *et al*. 1986, 1990; Galli *et al*. 1991), as well as striatal slice preparations (Roberts and Sharif 1978; Clow and Jhamandas 1989; Krebs *et al*. 1991a, 1991b; Iravani and Kruk 1996). Moreover, dopamine synthesis and glutamate-stimulated release via NMDA receptors has been demonstrated in striatal synaptosomes (Krebs *et al*. 1991b; Desce *et al*. 1992, 1994; Cheramy *et al*. 1994). However, the possible involvement of post-synaptic NMDA receptors cannot be ruled out. Post-synaptic NMDA receptors could enhance dopamine release by affecting synaptic ion concentrations or by stimulating release of nitric oxide by the post-synaptic neuron (Zetterstrom *et al*. 1988; Hurd and Ungerstedt 1989; Westerink *et al*. 1989; Hanbauer *et al*. 1992; Keefe *et al.* 1993). Alternatively, NMDA receptors located on striatal GABAergic or cholinergic interneurons could also enhance dopamine release by multisynaptic activation of nigral dopaminergic neurons (Whitehead *et al*. 2001).

We find that NMDA administration into the striatum through the microdialysis probe increases extracellular dopamine levels, consistent with previous results *in vitro* (Roberts and Sharif 1978; Roberts and Anderson 1979; Marien *et al*. 1983) and *in vivo* (Cheramy *et al*. 1986; Moghaddam *et al*. 1990; Keefe *et al*. 1992). The NMDA-induced release of dopamine is blocked by D-AP5, a competitive inhibitor of NMDA receptors, as has been observed previously (Marek *et al*. 1992; Whitton *et al*. 1994). These results are consistent with previous observations that intrastriatal administration of a glutamate re-uptake blocker increases extracellular dopamine in the neostriatum and that this effect is attenuated by NMDA antagonists (Segovia *et al*. 1997). Taken together, the results strongly support the hypothesis that both systemic and intrastriatal administration of 3α5βHS decrease NMDA-induced dopamine release (as measured by microdialysis) via negative modulation of NMDA receptors.

The present behavioral and neurochemical results indicate that peripherally administered 3α5βHS negatively modulates NMDA receptors in the CNS. Pharmacological manipulation of glutamatergic transmission by 3α5βHS could be a new strategy for the treatment of disorders that are characterized at least in part by the overactivation of these receptors. NMDA receptors are hypothesized to play a role in neurological and psychiatric disorders such as epilepsy (Herron *et al*. 1985; Dingledine *et al*. 1986), schizophrenia (Carlsson and Carlsson 1990; Wachtel and Turski 1990), excitotoxicity (Olney *et al*. 1981), addiction (Robinson and Berridge 1993; Pierce *et al*. 1997; Wolf 1998), and Parkinson's disease (Carlsson and Carlsson 1990). While NMDA receptor antagonists show promise in animal models of these

disorders, this drug class produces side-effects including ataxia (Carlsson and Carlsson 1989), hyperlocomotion (Koek *et al*. 1988), hallucinations (Lahti *et al*. 1995), and deficits in learning and memory (Morris *et al*. 1986). It is hoped that the development of negative modulators of NMDA receptors, such as 3α5βHS, may confer therapeutic benefit without the undesirable side-effects associated with NMDA receptor antagonists.

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References

Authier N., Dupuis E., Kwasiborski A., Eschalier A. and Coudore F. (2002) Behavioural assessment of dimethylsulfoxide neurotoxicity in rats. *Toxicol. Lett.* **132**, 117–121.

Baulieu E. E. (1998) Neurosteroids: a novel function of the brain. *Psychoneuroendocrinology* **23**, 963–987.

Bowlby M. (1993) Pregnenolone sulfate potentiation of *N*-methyl-D-aspartate receptor channels in hippocampal neurons. *Mol. Pharmacol.* **43**, 813–819.

Carlsson M. and Carlsson A. (1989) The NMDA antagonist MK-801 causes marked locomotor stimulation in monoamine-depleted mice. *J. Neural Transm.* **75**, 221–226.

Carlsson M. and Carlsson A. (1990) Interactions between glutamatergic and monoaminergic systems within the basal ganglia: implications for schizophrenia and Parkinson's disease. *Trends Neurosci.* **13**, 272–276.

Cheramy A., Romo R., Godehen G., Baruch P. and Glowinsky J. (1986) *In vivo* pre-synaptic control of dopamine release in the cat caudate nucleus. Facilitatory or inhibitory influence of glutamate. *Neuroscience* **19**, 1081–1090.

Cheramy A., Barbeito L., Godeheu G., Desce J. M., Pittaluga A., Galli T., Artaud F. and Glowinski J. (1990) Respective contributions of neuronal activity and pre-synaptic mechanisms in the control of the *in vivo* release of dopamine. *J. Neural Transm. Suppl.* **29**, 183–193.

Cheramy A., Desce J. M., Godeheu G. and Glowinski J. (1994) Pre-synaptic control of dopamine synthesis and release by excitatory amino acids in rat striatal synaptosomes. *Neurochem. Int.* **25**, 145–154.

Clow D. W. and Jhamandas K. (1989) Characterization of l-glutamate action on the release of endogenous dopamine from the rat caudate-putamen. *J. Pharmacol. Exp. Ther.* **248**, 722–728.

Desce J. M., Godeheu G., Galli T., Artaud F., Cheramy A. and Glowinski J. (1992) L-Glutamate-evoked release of dopamine from synaptosomes of the rat striatum: involvement of AMPA and *N*-methyl-D-aspartate receptors. *Neuroscience* **47**, 333–339.

Desce J. M., Godeheu G., Galli T., Glowinski J. and Cheramy A. (1994) Opposite pre-synaptic regulations by glutamte through NMDA receptors of dopamine synthesis and release in rat striatal synaptosomes. *Brain Res.* **640**, 205–214.

Dingledine R., Hynes M. A. and King G. L. (1986) Involvement of *N*-methyl-D-aspartate receptors in epileptiform bursting in the rat hippocampal slice. *J. Physiol.* **380**, 175–189.

Galli T., Godeheu G., Artaud F., Desce J. M., Pittaluga A., Barbeito L., Glowsinki J. and Cheramy A. (1991) Specific role of *N*-acetyl-aspartyl-glutamate in the *in vivo* regulation of dopamine release from dendrites and nerve terminals of nigrostriatal dopaminergic neurons in the cat. *Neuroscience* **42**, 19–28.

Greig N. H., Sweeney D. J. and Rapoport S. I. (1985) Inability of dimethyl sulfoxide to increase brain uptake of water-soluble compounds: implications to chemotherapy for brain tumors. *Cancer Treat. Rep.* **69**, 305–312.

Hanbauer I., Wink D., Osawa Y., Edelman G. M. and Gally J. A. (1992) Role of nitric oxide in NMDA-evoked release of [^{3}H]-dopamine from striatal slices. *Neuroreport* **3**, 409–412.

Herron C. E., Lester R. A., Coan E. J. and Collingridge G. L. (1985) Intracellular demonstration of an *N*-methyl-D-aspartate receptor mediated component of synaptic transmission in the rat hippocampus. *Neurosci. Lett.* **60**, 19–23.

Hurd Y. L. and Ungerstedt U. (1989) *In vivo* neurochemical profile of dopamine uptake inhibitors and releasers in rat caudate-putamen. *Eur. J. Pharmacol.* **166**, 251–260.

Iravani M. M. and Kruk Z. L. (1996) Real-time effects of *N*-methyl-D-aspartic acid on dopamine release in slices of rat caudate putamen: a study using fast cyclic voltammetry. *J. Neurochem.* **66**, 1076–1085.

Irwin R. P., Maragakis N. J., Rogawski M. A., Purdy R. H., Farb D. H. and Paul S. M. (1992) Pregnenolone sulfate augments NMDA receptor medicated increases in intracellular Ca^{2+} in cultured rat hippocampal neurons. *Neurosci. Lett.* **141**, 30–34.

Irwin R. P., Lin S. Z., Rogawski M. A., Purdy R. H. and Paul S. M. (1994) Steroid potentiation and inhibition of *N*-methyl-D-aspartate receptor-mediated intracellular Ca^{2+} responses: structure activity studies. *J. Pharmacol. Exp. Ther.* **271**, 677–682.

Keefe K. A., Zigmond M. J. and Abercrombie E. D. (1992) Extracellular dopamine in striatum: influence of nerve impulse activity in medial forebrain bundle and local glutamatergic input. *Neuroscience* **47**, 325–332.

Keefe K. A., Zigmond M. J. and Abercrombie E. D. (1993) *In vivo* regulation of extracellular dopamine in the neostriatum: influence of impulse activity and local excitatory amino acids. *J. Neural Transm. General Sect.* **91**, 223–240.

Koek W., Woods J. H. and Winger G. D. (1988) MK-801, a proposed noncompetitive antagonist of excitatory amino acid neurotransmission, produces phencyclidine-like behavioral effects in pigeons, rats and rhesus monkeys. *J. Pharmacol. Exp. Ther.* **245**, 969–974.

Krebs M. O., Desce J. M., Kemel M. L., Gauchy C., Godeheu G., Cheramy A. and Glowinski J. (1991a) Glutamatergic control of dopamine release in the rat striatum: evidence for pre-synaptic *N*-methyl-D-aspartate receptors on dopaminergic nerve terminals. *J. Neurochem.* **56**, 81–85.

Krebs M. O., Trovero F., Desban M., Gauchy C., Glowinski J. and Kemel M. L. (1991b) Distinct pre-synaptic regulation of dopamine release through NMDA receptors in striosome- and matrix-enriched areas of rat striatum. *J. Neurosci.* **11**, 1256–1262.

Kuppenbender K. D., Standaert D. G., Feuerstein T. J., Penney J. B. Jr, Young A. B. and Landwehrmeyer G. B. (2000) Expression of NMDA receptor subunit mRNAs in neurochemically identified projection and interneurons in the human striatum. *J. Comp. Neurol.* **419**, 407–421.

Lahti A. C., Koffel B., LaPorte D. and Tamminga C. A. (1995) Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. *Neuropsychopharmacology* **13**, 9–19.

Landwehrmeyer G. B., Standaert D. G., Testa C. M., Penney J. B. Jr and Young A. B. (1995) NMDA receptor subunit mRNA expression by projection neurons and interneurons in rat striatum. *J. Neurosci.* **15**, 5297–5307.

Malayev A., Gibbs T. T. and Farb D. H. (2002) Inhibition of the NMDA response by pregnenolone sulphate reveals subtype selective modulation of NMDA receptors by sulphated steroids. *Br. J. Pharmacol.* **135**, 901–909.

Marek P., Chapman C. D. and Howard S. (1992) The effect of phencyclidine and DL-2-amino-5-phosphonovaleric acid on *N*-methyl-D-aspartic acid induced changes in extracellular concentration of dopamine and DOPAC in the rat neostriatum. *Neuropharmacology* **31**, 123–127.

Marien M., Brien J. and Jhamandas K. (1983) Regional release of [^{3}H]dopamine from rat brain in vitro: effects of opioids on release induced by potassium, nicotine, and l-glutamic acid. *Can J. Physiol. Pharmacol.* **61**, 43–60.

Martinez-Fong D., Rosales M. G., Gongora-Alfaro J. L., Hernandez S. and Aceves J. (1992) NMDA receptors mediates dopamine release in the striatum of unanesthetized rats as measured by brain microdialysis. *Brain Res.* **595**, 309–315.

Moghaddam B., Roth R. H. and Bunney B. S. (1990) Characterization of dopamine release in the rat medial pre-frontal cortex as assessed by *in vivo* microdialysis: comparison to the striatum. *Neuroscience* **36**, 669–676.

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

Neuwelt E. A., Barnett P., Barranger J., McCormick C., Pagel M. and Frenkel E. (1983) Inability of dimethyl sulfoxide and 5-fluorouracil to open the blood-brain barrier. *Neurosurgery* **12**, 29–34.

Olney J. W., Labruyere J., Collins J. F. and Curry K. (1981) D-amino-phosphonovalerate is 100-fold more powerful than D-α- aminoadipate in blocking *N*-methyl-aspartate neurotoxicity. *Brain Res.* **221**, 207–210.

Ossowska K. and Wolfarth S. (1995) Stimulation of glutamate receptors in the intermediate/caudal striatum induces contralateral turning. *Eur J. Pharmacol.* **273**, 89–97.

Park-Chung M., Wu F. S. and Farb D. H. (1994) 3α-Hydroxy-5β-pregnan-20-one sulfate: a negative modulator at the NMDA receptor. *Mol. Pharmacol.* **46**, 146–150.

Park-Chung M., Wu F. S., Purdy R. H., Malayev A. A., Gibbs T. T. and Farb D. H. (1997) Distinct sites for inverse modulation of NMDA receptors by sulfated steroids. *Mol. Pharmacol.* **52**, 1113–1123.

Paul S. M. and Purdy R. H. (1992) Neuroactive steroids. *FASEB J.* **6**, 2311–2322.

Paxinos G. and Watson C. (1997) *The Rat Brain in Stereotaxic Coordinates,* 3rd Edn. New York: Academic Press.

Pierce R. C., Meil W. M. and Kalivas P. W. (1997) The NMDA antagonist, dizocilpine, enhances cocaine reinforcement without influencing mesoaccumbens dopamine transmission. *Psychopharmacology* **133**, 188–195.

Rabow L. E., Russek S. J. and Farb D. H. (1995) From ion currents to genomic analysis: recent advances in GABAA receptor research. *Synapse* **21**, 189–274.

Riederer P., Lange K. W., Kornhuber J. and Danielczyk W. (1992) Glutamatergic–dopaminergic balance in the brain. Its importance in motor disorders and schizophrenia. *Arzneimittelforschung* **42**, 265–268.

Roberts P. J. and Anderson S. D. (1979) Stimulatory effect of l-glutamate and related amino acids on [^{3}H]dopamine release from rat striatum: an *in vitro* model for glutamate actions. *J. Neurochem.* **32**, 1539–1545.

Roberts P. J. and Sharif N. A. (1978) Effects of l-glutamate and related amino acids upon the release of [^{3}H]dopamine from rat striatal slices. *Brain Res.* **157**, 391–395.

Robinson T. E. and Berridge K. C. (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res. Rev.* **18**, 247–291.

Schmidt W. J., Bubser M. and Hauber W. (1992) Behavioural pharmacology of glutamate in the basal ganglia. *J. Neural Transm. Suppl.* **38**, 65–89.

Segovia G., Porras A. and Mora F. (1997) Effects of 4-aminopyridine on extracellular concentrations of glutamate in striatum of the freely moving rat. *Neurochem. Res.* **22**, 1491–1497.

Sesack S. R. and Pickel V. M. (1990) In the medial nucleus accumbens, hippocampal and catecholaminergic terminals converge on spiny neurons and are in apposition to each other. *Brain Res.* **527**, 266–272.

Taepavarapruk P., Floresco S. B. and Phillips A. G. (2000) Hyperlocomotion and increased dopamine efflux in the rat nucleus accumbens evoked by electrical stimulation of the ventral subiculum: role of ionotropic glutamate and dopamine D1 receptors. *Psychopharmacology* **151**, 242–251.

Wachtel H. and Turski L. (1990) Glutamate: a new target in schizophrenia? *Trends Pharmacol. Sci.* **11**, 219–220.

Walters A., Jackson-Lewis V. and Fahn S. (1984) Little effect of dimethyl sulfoxide on blood-brain barrier to dopamine. *Experientia* **40**, 859–861.

Weaver C. E., Jr Marek P., Park-Chung M., Tam S. W. and Farb D. H. (1997) Neuroprotective activity of a new class of steroidal inhibitors of the *N*-methyl-D-aspartate receptor. *Proc. Natl Acad. Sci. USA* **94**, 10450–10454.

Weaver C. E. Jr, Wu F.-S., Gibbs T. T. and Farb D. H. (1998) Pregnenolone sulfate exacerbates NMDA-induced death of hippocampal neurons. *Brain Res.* **803**, 129–136.

Westerink B. H. C., Hofsteede R. M., Tuntler J. and de Vries J. B. (1989) Use of calcium antagonism for the characterization of drug-evoked dopamine release from the brain of conscious rats determined by microdialysis. *J. Neurochem.* **52**, 722–729.

Whitehead K. J., Rose S. and Jenner P. (2001) Involvement of intrinsic cholinergic and GABAergic innervation in the effect of NMDA on striatal dopamine efflux and metabolism as assessed by microdialysis studies in freely moving rats. *Eur. J. Neurosci.* **14**, 851–860.

Whitton P. S., Maione S., Biggs C. S. and Fowler L. J. (1994) *N*-methyl-D-aspartate receptors modulate extracellular dopamine concentration and metabolism in rat hippocampus and striatum in vivo. *Brain Res.* **635**, 312–316.

Wolf M. E. (1998) The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. *Prog. Neurobiol.* **54**, 679–720.

Wong M. and Moss R. L. (1994) Patch-clamp analysis of direct steroidal modulation of glutamate receptor-channels. *J. Neuroendocrinol.* **6**, 347–355.

Wu F. S., Gibbs T. T. and Farb D. H. (1991) Pregnenolone sulfate: a positive allosteric modulator at the *N*-methyl-D-aspartate receptor. *Mol. Pharmacol.* **40**, 333–336.

Wu M., Brudzynski S. M. and Mogenson G. J. (1993) Functional interaction of dopamine and glutamate in the nucleus accumbens in the regulation of locomotion. *Can. J. Physiol. Pharmacol* **71**, 407–413.

Yaghoubi N., Malayev A., Russek S. J., Gibbs T. T. and Farb D. H. (1998) Neurosteroid modulation of recombinant ionotropic glutamate receptors. *Brain Res.* **803**, 153–160.

Zetterstrom T., Sharp T., Collin A. K. and Ungerstedt U. (1988) In vivo measurement of extracellular dopamine and DOPAC in rat striatum after various dopamine-releasing drugs; implications for the origin of extracellular DOPAC. *Eur. J. Pharmacol.* **148**, 327–334.