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Neurosteroids and reward: allopregnanolone produces a conditioned place aversion in rats

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Abstract

The neurosteroid 3α-hydroxy-5α-pregnan-20-one (allopregnanolone) has been reported to have rewarding properties in mice tested for place conditioning. Another study found that allopregnanolone reduced dopamine (DA) output in the nucleus accumbens (NAc) of rats. As many rewarding stimuli increase accumbens DA, these results may appear contradictory. Thus, the present study examined the rewarding properties of allopregnanolone in rats tested for place conditioning using an unbiased conditioning procedure. In control studies, a place preference was observed following conditioning with intraperitoneal (2.0 mg/kg) or intracerebroventricular (i.c.v.) (100 μg/0.5 μl) amphetamine. Conditioning with i.c.v. allopregnanolone produced a significant aversion at a dose of 5.0 μg (in 5.0 μl) and a near aversion at 25.0 μg (in 8.3 μl); doses of 0 μg (i.e., vehicle alone, in 10 μl) or 30.0 μg (in 10 μl) produced little effect on place preference. During conditioning, locomotor activity was stimulated by amphetamine using either route of administration, but allopregnanolone had no significant main effect on locomotor activity. Thus, there was a dissociation between the effects of drugs on locomotor activity vs. place conditioning. Results show that i.c.v. amphetamine produces a place preference, whereas allopregnanolone produces either no effect or an aversion, depending on the dose.

Keywords: Allopregnanolone; Amphetamine; Dopamine; Locomotor activity; Neurosteroid; Place conditioning

1. Introduction

For many years, it has been known that steroids exert a genomic effect on the central nervous system. However, recent research has shown that steroids can also have a non-genomic effect. Thus, it has been found that the brain can synthesize steroids that influence, for example, the γ-aminobutyric acid ($GABA_A$) receptor in the central nervous system; these agents have been termed neurosteroids [14].

Neurosteroids may influence reward-related learning. Allopregnanolone, a neurosteroid metabolite of progesterone that is produced in brain glial cells and peripheral tissues, enhances GABAergic neurotransmission by augmenting GABA-stimulated Cl^- influx [16]. In studies of place conditioning in mice, systemic allopregnanolone produced an increase in locomotor activity during conditioning and a dose-dependent preference for the place previously associated with it [9]. The benzodiazepine diazepam also enhances GABAergic neurotransmission and produces a place preference [7,22,23].

Other findings may appear to be in conflict with the finding that allopregnanolone or diazepam produce reward. Motzo et al. [18], using intracerebral microdialysis, observed that intracerebroventricular (i.c.v.) allopregnanolone inhibits basal and stress-induced dopamine (DA) release in both the rat prefrontal cortex and nucleus accumbens (NAc). NAc DA release is also decreased by diazepam [8,12,17]. Considerable evidence shows that DA release in the NAc is necessary for reward (for review, see Refs. [13,20]). For example, Carr and White [4,5], examining brain regions where injections of the DA agonist amphetamine might produce reward, found that the NAc was the only region

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where injections resulted in a place preference. Others have shown that intra-NAc injections of DA receptor antagonists block place conditioning produced by systemic amphetamine (for review, see Refs. [11,24]). Thus, the reports that allopregnanolone and diazepam produce a place preference and decrease the release of DA in the NAc would appear to be contradictory.

The apparent contradiction in the results from studies of allopregnanolone in place conditioning and intracerebral microdialysis may have arisen because of methodological differences between the two studies. Besides the species difference, the place-conditioning study used systemic administration, whereas the dialysis study used i.c.v. administration of allopregnanolone. To evaluate the possible influence of some of these variables, we undertook this study to assess place conditioning in rats (instead of mice) following i.c.v. (instead of systemic) injections of allopregnanolone. Based on the microdialysis results of Motzo et al. [18], we hypothesized that allopregnanolone would not produce a place preference. We chose our doses of allopregnanolone based on the Motzo et al. [18] study; thus, 5.0 μg i.c.v. was chosen as a dose that produced little effect on DA release, and 25.0 μg was chosen as a dose that would be expected to produce a large decrease in DA release. After we saw a place aversion with both of these doses, we chose 30.0 μg to extend the dose–response curve; limits in solubility prevented us from testing higher doses. As a positive control, we also assessed place conditioning following systemic or i.c.v. amphetamine.

2. Materials and methods

Use of the rats in this study was in accordance with the Animals for Research Act, the Guidelines of the Canadian Council on Animal Care and relevant University policies, and was approved by the Queen's University Animal Care Committee.

2.1. Subjects

Male Wistar rats (Charles River, Canada) weighing 200–250 g upon arrival were housed in pairs in a climatically controlled (21°C) colony room, with a 12-h light (07:00–19:00 hours)/dark cycle. Rodent chow (Richmond standard lab diet #5001) and water were available in the home cages at all times. The rats were handled on a daily basis for the first week after arrival before the beginning of experimental procedures.

2.2. Apparatus

Place conditioning and locomotor activity were monitored in four similar rectangular boxes (84×27×36 cm high) constructed of wooden sides and removable Plexiglas covers. Each box consisted of two chambers joined by a small tunnel ($8 \times 8 \times 6$ cm high) that could be blocked by the insertion of Plexiglas guillotine doors. The chambers differed in wall pattern and floor design. In two of the conditioning boxes, one chamber had unpainted (brown) urethane-sealed walls and a wire mesh floor (1×1 cm), while the other chamber had black and white vertically striped walls (stripes were 1 cm wide), and a floor consisting of wire rods spaced 1 cm apart. In the other two chambers, the floor and wall pairings were reversed and, in the four boxes, the wall–floor combinations were arranged such that among them each of the wall types and floor types appeared on both the left and right sides of the apparatus. Each box was housed in an outer shell that was insulated with sound attenuating Styrofoam, illuminated by a 7.5-W light, and ventilated with a small fan.

Each box was equipped with six pairs of infrared photosensors: two located 5 cm above the floor in each chamber, two located 3 cm above the floor of the tunnel, and a signal amplifier unit. Each set of photosensors was connected to an Experiment Controller (EC Board), which sampled the photosensors repeatedly to monitor sensor interruptions. Sensor counts were recorded and stored in 5-min bins for each day of each experiment. Data from the EC Boards were then dumped to a Macintosh computer, which served as the host. For further details, see Brockwell et al. [1].

2.3. Surgery

Anesthesia was induced with Halothane at a flow rate of 4%, and then the flow rate was steadily decreased from 3% to 1.5% during surgery, as required, to maintain a stable respiratory rate. After placing rats in a stereotaxic frame, presurgery injections of the analgesic Buprenorphine (0.06 mg) were given. A single stainless-steel cannula (0.6 mm outer diameter) was implanted into the lateral ventricle using the coordinates: 1.0 mm posterior to bregma, 1.5 mm lateral to midline, and 3.0 mm ventral to the surface of the dura, with the skull kept level between lambda and bregma [19]. The cannulae were placed alternately into the right or left ventricle and were anchored to the skull using four screws and dental cement. The rats then received six intradermal injections of Marcaine, a local anesthetic. Before returning rats to a recovery cage, a subcutaneous injection of Ringer's solution (1.0 ml) was administered, and Polysporin was applied to the area of the wound. Rats were given 1 week to recover before beginning preconditioning.

2.4. Injection procedures

In the first experiment, the rats were injected intraperitoneally with 2.0 mg/kg amphetamine during the drug sessions (odd days) and were injected with 0.9% saline (1.0 ml/kg) during the vehicle sessions (even days; see below). For the i.c.v. amphetamine (100 μg in 0.5 μl) and

allopregnanolone (0 (i.e., cyclodextrin alone; see below), 5.0, 25.0, or 30.0 μg in 10.0, 5.0, 8.3, or 10.0 μl, respectively) experiments, infusions were made using injection cannulae (0.3 mm outer diameter) that were 1.0 mm longer than the guides (ventral=4.0 mm). Injectors were connected via polyethylene tubing to a 10-μl Hamilton syringe mounted on a perfusion pump and the injection rate was 1.0 μl/min. After the injection, the injector was left in situ for 2 min to permit diffusion. On vehicle days, the volume was 0.5 μl for the i.c.v. amphetamine and 10, 5.0, 8.3, and 10 μl, respectively, for the allopregnanolone groups. For both drugs, the injections were given 5 min prior to the beginning of the conditioning session, and the rats were held in metal transporting cages during that time.

2.5. Drugs

For the first experiment, amphetamine sulfate (Smithkline Beecham Pharma) was dissolved in saline (0.9% NaCl) to a concentration of 2.0 mg/ml and injected i.p. at a volume of 1.0 ml/kg, yielding a dose of 2.0 mg/kg. The drug was mixed fresh every drug conditioning day. For the i.c.v. amphetamine experiment, a dose of 100 μg was used, and the drug was dissolved in saline to a concentration of 10.0 mg/50.0 μl, and a volume of 0.5 μl was injected. The drug was mixed fresh for every drug conditioning day. For the third set of experiments, doses of 0 (i.e., cyclodextrin alone), 5.0, 25.0, and 30.0 μg of allopregnanolone (Research Biochemicals International, Natick, MA) were used; the drug was dissolved to a concentration of either 1 or 3 mg/ml by sonication in saline, complexed with 45% 2-hydroxypropyl-β-cyclodextrin (cyclodextrin; Research Biochemicals International) for 4 h. The drug was then aliquoted and frozen. Volumes of 10.0, 5.0, 8.3, and 10.0 μl, respectively, were injected i.c.v. On vehicle injection days for the two amphetamine experiments, 0.9% saline was injected in the same volumes as the drug. The vehicle injections for the 5.0, 25.0, and 30.0 μg allopregnanolone experiments were 0.9% saline, complexed with 45% cyclodextrin, and were given at a volume of 5.0 μl. For the 0-μg dose of allopregnanolone, the vehicle (i.e., the substance injected on the nondrug side) was 0.9% saline injected in a volume of 10 μl.

2.6. Procedure

Behavioral testing lasted for 12 days, and consisted of three phases: preconditioning, conditioning, and testing. Experimental sessions were conducted at 24-h intervals during the light period of the light/dark cycle.

2.6.1. Preconditioning

This phase was included to habituate the rats to the conditioning boxes and provide a baseline measure of unconditioned chamber preference. During three 15-min sessions, rats were placed in one of the chambers (designated the start side), and allowed access to the entire box. The choice of start side (left or right) was counterbalanced across rats, but remained the same for each rat throughout the experiment.

2.6.2. Conditioning

During each of the eight 30-min conditioning sessions, animals were confined to one chamber by blocking the entrance to the tunnel. On odd-numbered sessions, animals were administered the drug and confined to one chamber. On even-numbered sessions, rats received the vehicle and were confined to the opposite chamber.

2.6.3. Testing

During the 15-min test sessions, animals in a drug-free state were placed in the start chamber and allowed access to the entire box.

Between each individual use of the apparatuses, the floors were cleaned and scrubbed with a 50:50 vinegar/water solution.

2.7. Histology

At the conclusion of behavioural studies, all operated rats were euthanized with carbon dioxide, and their brains were extracted and fixed in a 10% formalin solution. The brains then were blocked and 70-μm frozen sections were cut on a freezing-stage microtome. The slices were subsequently mounted on slides, stained with thionine, and verified histologically for cannulae placements by an observer who was naive to the behavioral results for individual animals. Positive cannula placements were defined by clear positioning of the cannula in the lateral ventricle, unambiguous penetration of the corpus callosum, and protrusion no further than the ventral limit of the ventricle. Rats displaying evidence of oedema or incorrect cannula placement were eliminated from the study. Therefore, 44 operated animals were included in the analyses.

2.8. Statistical analyses

The establishment of place conditioning was assessed for each experiment by comparing the mean time spent in the drug-paired chamber during preconditioning with the time spent there during the test. A paired sample t-test was used to analyze the data. For the amphetamine experiments, it was hypothesized that the animals would spend more time on the drug-paired side during the test session vs. the average of the preconditioning sessions; therefore, the t-value was tested against a p-value for a directional prediction ($\alpha=0.05$). The t-value for the allopregnanolone test was evaluated at a level of significance for a two-tailed test ($\alpha=0.05$).

Locomotor activity for each experiment was assessed by a two-way (treatment × bin) repeated-measures analysis of variance (ANOVA). This analysis compared: (1) treatment

— the number of sensor counts during vehicle conditioning to the number of sensor counts during drug conditioning (data were averaged over the 4 days of each treatment); and (2) bin — the number of sensor counts in each 5-min period of the four 30-min conditioning sessions averaged together. Significant effects were further analyzed with post hoc tests where appropriate.

3. Results

3.1. Place conditioning

Table 1 shows the mean (±SEM) time (s) spent, during the three preexposure sessions averaged together, in the side of the test apparatus that was paired with the drug during conditioning sessions, in the tunnel, and in the side of the apparatus that was paired with vehicle during conditioning sessions. Paired *t*-tests compared the time spent on the two sides for each group and revealed that none of the differences was significant (Table 1). Thus, there was no significant side bias for any group.

Mean (±SEM) differences between the amount of time spent on the drug-paired side averaged over the three preconditioning days and the value for the test day for both i.p. and i.c.v. amphetamine experiments are shown in Fig. 1. There was a clear preference for the drug-paired side in the test for the i.p. and i.c.v. amphetamine experiments. Statistical comparisons of the mean time spent on the drug-paired side during preconditioning days vs. the test day were significant for each experiment: $t(11)=4.52$, $p<0.005$ and $t(9)=2.09$, $p<0.05$, respectively. The results for the allopregnanolone experiments (Fig. 1) yielded no change in time spent on the drug-paired side for the vehicle (0-μg dose) experiment, $t(9)<1.0$, $p>>0.05$. There was a significant decrease in time spent on the drug-paired side from preconditioning to test, i.e., an aversion for the 5.0-μg dose, $t(6)=2.53$, $p<0.05$, a near significant aversion for the 25.0-μg dose, $t(8)=2.10$, $p<0.07$, and no change for the 30.0-μg dose, $t(7)<1.0$, $p>>0.05$.

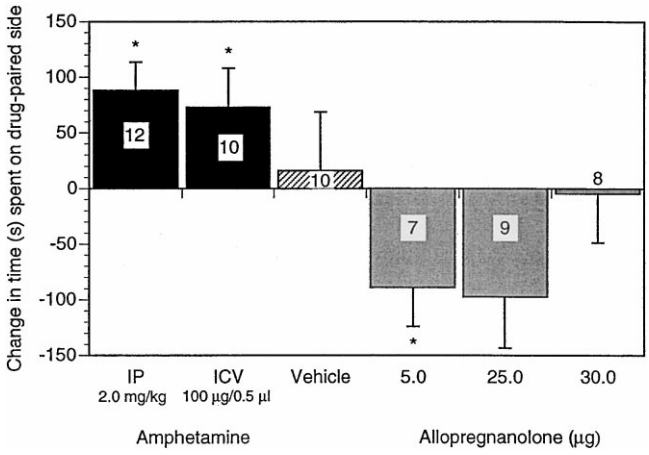

Fig. 1. Mean (+SEM) change in time (s) spent on the drug-paired side from preexposure (3 days averaged together) to test for groups receiving pairings with the drug-associated side of i.p. or i.c.v. amphetamine, or allopregnanolone doses of 0 (vehicle), 5.0, 25.0, or 30.0 μg in volumes of 10.0, 5.0, 8.3, or 10.0 μl. * Significant ($p<0.05$) change in time spent on the drug-paired side from preexposure to test.

3.2. Locomotor activity

Fig. 2A and B depicts the mean activity counts per minute during successive 5-min bins of time for the average of the drug and vehicle conditioning days. Days were averaged because there was no significant change in the magnitude of the response to amphetamine across the four conditioning days for either group. For the i.p. and i.c.v. amphetamine experiments, locomotor activity was elevated during drug conditioning days when compared to activity on vehicle conditioning days, $F(1, 11)=26.50$, $p<0.001$ and, $F(1, 9)=11.32$, $p<0.01$, respectively. In both cases, locomotor activity decreased across bins, $F(5, 55)=19.78$, $p<0.001$, and, $F(5, 45)=66.67$, $p<0.001$. In the i.p. experiment, the treatment × bin interaction was found to be significant, $F(5, 55)=6.08$, $p<0.001$. A Newman–Keuls post hoc test revealed that activity was elevated following amphetamine injection compared to the last five bins following vehicle injection.

Table 1
Mean (±SEM) time (s), spent during the three preexposure sessions averaged together in the side of the test apparatus that was paired with the drug during conditioning sessions (drug-paired chamber), in the tunnel, and in the side of the apparatus that was paired with vehicle during conditioning sessions (vehicle-paired chamber)

Condition	Mean time (s) in drug-paired chamber	Mean time (s) spent in tunnel	Mean time (s) in vehicle-paired chamber	Paired *t*-value (drug vs. vehicle)	Paired *p*-value
I.p. amphetamine ($n=12$)	438.6±20.5	64.5±6.2	396.9±16.6	−1.1	$p>0.05$
I.c.v. amphetamine ($n=10$)	419.9±28.8	46.0±5.1	434.1±27.0	0.3	$p>0.05$
Vehicle ($n=10$)	470.1±22.9	39.7±3.6	390.1±23.5	−1.6	$p>0.05$
5 μg allopregnanolone ($n=7$)	487.0±31.0	39.7±4.6	375.8±33.6	−1.8	$p>0.05$
25 μg allopregnanolone ($n=9$)	460.8±42.0	41.4±2.7	390.1±41.3	−0.8	$p>0.05$
30 μg allopregnanolone ($n=8$)	447.3±60.6	40.8±9.9	411.9±62.9	−0.3	$p>0.05$

The *p*-values and the results of paired *t*-tests comparing the two sides during pre-exposure sessions revealed no significant differences, showing that there was no significant side bias in any group.

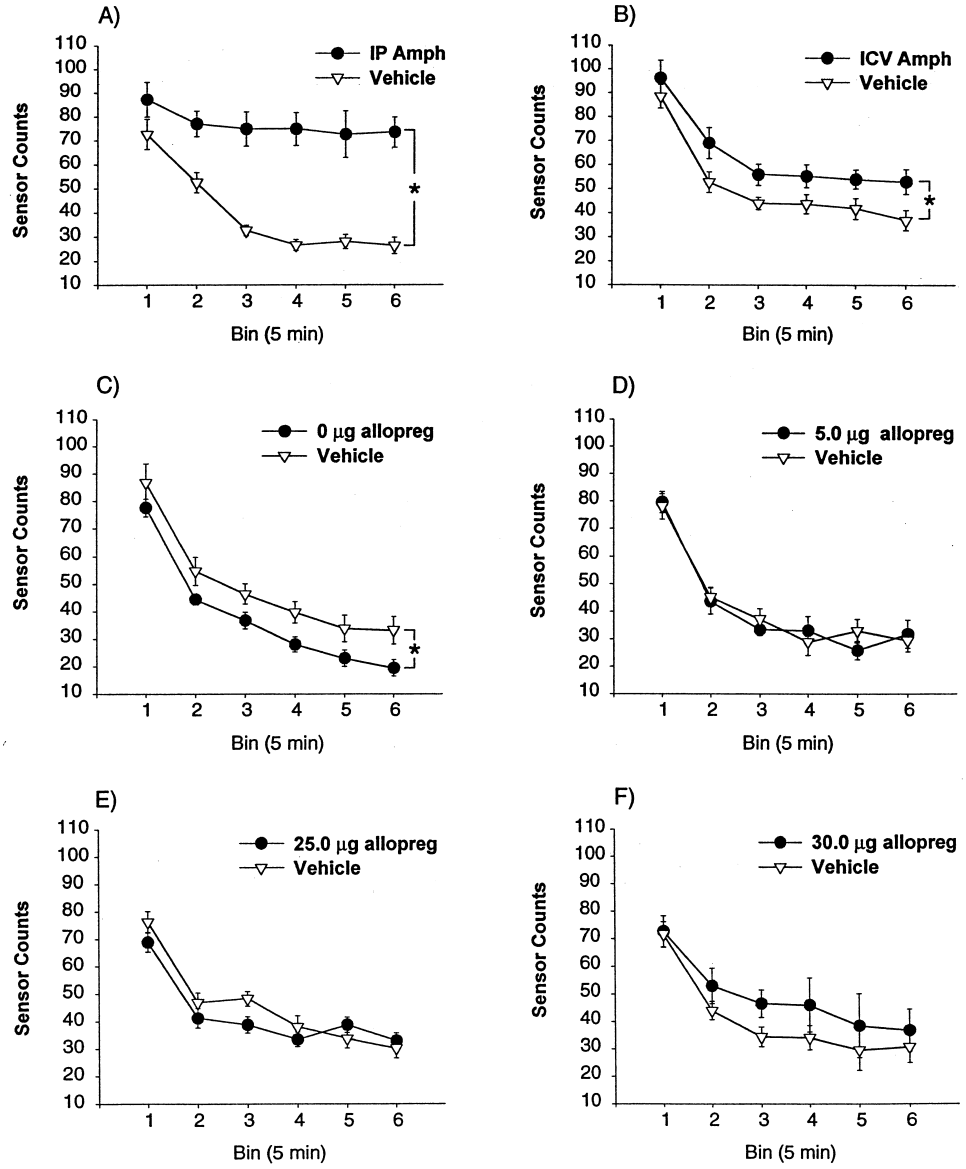

Fig. 2. Mean (±SEM) sensor counts per minute in 5-min bins for the 30-min conditioning days averaged together for the 4 days on the drug-paired side and for the 4 days on the vehicle-paired side. (A) I.p. amphetamine (20 mg/kg); (B) i.c.v. amphetamine (100 μg/0.5 μl); (C–F) i.c.v. allopregnanolone (allopreg) doses of 0 (cyclodextrin alone), 5.0, 25.0, and 30.0 μg in volumes of 10.0, 5.0, 8.3, and 10.0 μl.

For the groups receiving allopregnanolone doses of either 0 (i.e., cyclodextrin alone), 5.0, 25.0, or 30.0 μg (Fig. 2C–F), data were also averaged across days because there was no significant change in the magnitude of the response to the drug across the four conditioning days for any group but the 30-μg dose group. For this group, the mean activity (cpm) across days was 44.9, 42.4, 43.5, and 64.5; this effect was significant, $F(4, 21)=4.20$, $p<0.05$, and appeared to be due to the large increase in activity of this group on the last conditioning day. There was a significant reduction in locomotor activity across bins for each group: $F(5, 45)=70.07$, $p<0.001$; $F(5, 30)=47.97$, $p<0.001$; $F(5, 35)=76.67$, $p<0.001$; $F(5, 35)=22.72$, $p<0.001$, respectively. There was a significant effect of cyclodextrin alone, but not 5.0, 25.0, or 30.0 μg allopregnanolone on activity compared to vehicle: $F(1, 9)=8.53$, $p<0.05$; $F(1, 6)=0.12$, $p<0.05$; $F(1, 7)=1.04$, $p<0.05$; $F(1, 7)=1.32$, $p<0.05$, respectively. The treatment × bin interaction was found to be significant for the 25.0-μg dose of allopregnanolone, $F(5, 35)=3.58$, $p<0.01$; this appeared to reflect the crossing over of the two treatment conditions in bins 5 and 6 compared to bins 1–4. It is noteworthy that although the 30.0-μg dose group of allopregnanolone was observed to show a significantly elevated locomotor response to the drug on the fourth injection day compared to the first 3 injection days, the activity level of this group averaged over the 4 conditioning days when injected with drug was not significantly higher than the activity level averaged over the 4 vehicle days. Thus, although the response to 30-μg allopregnano-

lone was elevated on the fourth conditioning day, this elevation was not sufficient to produce an overall significant increase compared to vehicle levels.

4. Discussion

The results can be summarized as follows: amphetamine injected i.p. produced a place preference, as indexed by a significant increase in the time spent in the drug-paired compartment in the test phase compared to the preconditioning days. I.p. amphetamine also produced a clear and significant increase in locomotor activity. I.c.v. amphetamine produced similar effects. Allopregnanolone, injected i.c.v., produced a significant aversion with the 5.0-μg dose, a near significant aversion at the 25.0-μg dose and almost no change for the 30.0-μg dose. None of these doses produced significant main effects on activity during conditioning. When administered alone, the vehicle for allopregnanolone (i.e., cyclodextrin) produced almost no change in time spent on the drug-paired side.

The results of the i.p. experiment corroborate many other findings that amphetamine produces a place preference (e.g., for review, see Ref. [10]). In a microdialysis experiment, Carboni et al. [3] demonstrated that amphetamine increases the output of DA in the NAc, and argued that this is consistent with the ability of amphetamine to stimulate DA release by displacing the neurotransmitter from an intracellular compartment. Thus, the effect of amphetamine on place-preference conditioning observed here is probably due to stimulation of DA release in the NAc.

To our knowledge, the i.c.v. amphetamine experiment is the first to demonstrate the conditioned place-preference effect using the i.c.v. route of administration. An effect was expected based on previous findings that amphetamine injected systemically [24] or directly into the NAc [4,5] produces a place preference. This confirmed that substances that have been shown to produce a place preference when given by the systemic or local injection route can produce a preference when given i.c.v.

The place aversion results for the allopregnanolone doses used here contradict the findings of Finn et al. [9], who observed a significant place preference after allopregnanolone was injected systemically into mice. At the three doses tested here, we did not observe that allopregnanolone produces a significant place preference. Indeed, allopregnanolone produced an aversion at one dose. A dose of 5.0 μg produced a significant aversion, and 25.0 μg produced a similar effect that was near significance. The apparent contradiction in the results of the study of Finn et al. [9] and the present study may have arisen because of methodological differences between the two studies. Besides the species difference (mice vs. rats, respectively), Finn et al. [9] used systemic administration, whereas the present study used i.c.v. administration of allopregnanolone. The possible importance of these and other methodological variables (e.g., number of preexposure sessions, duration of sessions, etc.) will have to await further study.

An allopregnanolone dose of 30.0 μg produced little change in time spent on the drug-paired side. At the 30.0-μg dose, allopregnanolone may be producing central effects that counteract the aversive effects of lower doses, although the nature of these additional effects is not known. In agreement with the present observation of a "U"-shaped dose–response curve for place aversion learning produced by the neurosteroid allopregnanolone, others have reported that the anti-GABA, pro-NMDA neurosteroid pregnanolone similarly produces a "U"-shaped dose–response curve for its reversal of scopolamine-induced discrimination learning deficits [15]. At present, the mechanisms underlying the changes in effectiveness of these neurosteroids at higher doses are not known.

In their microdialysis study, Motzo et al. [16] found that allopregnanolone (5–15 μg in 5.0 μl of cyclodextrin vehicle) reduced the basal release of DA in the prefrontal cortex and NAc. They suggested that DA release is modulated by $GABA_A$ receptors localized in the ventral tegmental area, cerebral cortex, and NAc. As allopregnanolone enhances GABAergic neurotransmission, it would decrease DA release through its action at these receptors. Because DA has been found to be critical for place conditioning, allopregnanolone may have produced an aversion through this mechanism. However, the finding that diazepam similarly decreases NAc DA release [8,12,17] but produces a place preference [7,22,23] makes this explanation unlikely.

The results for locomotor activity are typical for amphetamine. Thus, elevated locomotor activity frequently has been observed following i.p. (e.g., Refs. [6,21]) or i.c.v. amphetamine [2], indicating the locomotor stimulant effect of this drug. Wise and Bozarth [25] have related this psychomotor stimulant action of amphetamine to its ability to produce rewarding effects. Repeated injections of amphetamine in the same test environment often have been reported to lead to sensitization to the drug effect, a larger stimulant effect being seen from injection to injection. Sensitization was not observed in the present experiment. Whether this observation was the result of the small number of injection days (4), the place conditioning protocol with alternating days of vehicle and drug injection, or some other differences between place conditioning vs. sensitization procedures is not clear from the present results.

Activity was lower on the drug-paired side for the 0-μg allopregnanolone (cyclodextrin alone) injection. The 0-μg injection experiment compared the effects of the cyclodextrin vehicle alone (0 μg in 10-μl dose) with saline (10 μl), and showed that locomotor activity was significantly reduced on the side associated with cyclodextrin in 10 μl. We know of no previous reports of locomotor effects with i.c.v. cyclodextrin and the mechanisms underlying this observation remain unclear. However, in the three allopregnanolone

dose groups, cyclodextrin was injected on both sides during conditioning, either alone (vehicle-paired side) or with the drug added (drug-paired side). Thus, any activity suppressant effects of cyclodextrin should have been similar on both sides. The observation of no significant main effects of allopregnanolone at any dose on locomotor activity, therefore, should be unrelated to the vehicle or the volume of the injections. Finn et al. [9] found that allopregnanolone i.p. had stimulant effects, and that the dose that produced the greatest stimulant effect also produced the largest place preference. Our results show no significant main effect of i.c.v. allopregnanolone on locomotor activity at a dose that produced a significant place aversion, showing a clear dissociation of the locomotor and place conditioning effects of this drug.

5. Conclusion

These experiments show that both i.p. and i.c.v. injections of amphetamine produce a conditioned place preference and elevated locomotor activity in rats. Contrary to previous findings, the neurosteroid allopregnanolone, when injected i.c.v., does not produce a place preference and does not significantly affect locomotor activity. Rather, allopregnanolone produces a place aversion or no effect depending on the dose. It will be the chore of future studies to determine the different sites of action of systemic [9] vs. i.c.v. (present study) allopregnanolone that are responsible for their differing effects on place conditioning and locomotor activity when given by the i.p. vs. i.c.v. route of administration.

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