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Amphetamine Infused Into the Ventrolateral Striatum Produces Oral Stereotypies and Conditioned Place Preference

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BAKER, D. A., S. E. SPECIO, L. T. L. TRAN-NGUYEN AND J. L. NEISEWANDER. *Amphetamine infused into the ventrolateral striatum produces oral stereotypies and conditioned place preference.* PHARMACOL BIOCHEM BEHAV **61**(1) 107–111, 1998.—The effects of amphetamine infused into the ventrolateral striatum (VLS) on locomotion, stereotypies, and conditioned place preference (CPP) were investigated. Five 2-day conditioning trials were conducted over 10 consecutive days. On 1 day of each trial, animals received an infusion of amphetamine (0, 2.5, 5, 10, or 20 mg/0.5 ml/side) and were placed into a distinct compartment for 30 min. On the other day, animals received sham intracranial infusions and were placed into a different compartment for 30 min. Locomotion and stereotypies were assessed following the first and last amphetamine infusions. CPP was assessed the day following the last conditioning trial. Intra-VLS infusions of amphetamine did not alter sniffing or locomotion. Acute administration of amphetamine into the VLS dose dependently produced oral stereotypies, however, tolerance developed to this effect following repeated administrations. Also, intra-VLS infusions of amphetamine dose dependently produced CPP. These results suggest that the VLS is involved in amphetamine-induced oral stereotypies and reward. © 1998 Elsevier Science Inc.

Amphetamine Oral stereotypies Locomotion Reward Conditioned place preference Ventrolateral striatum

THE striatum has traditionally been divided into two functionally distinct regions: the ventral or ventromedial striatum, and the dorsal or dorsolateral striatum. The ventral striatum typically includes the nucleus accumbens (NAc), the olfactory tubercle [OT; (19,26)], and occasionally the ventromedial caudate-putamen [CPu; (30)]. Anatomical tracing studies suggest that the ventral striatum, particularly the NAc, may integrate motor and limbic processes because it receives dopaminergic projections from the ventral tegmental area [VTA; (4,29)] and limbic projections from the amygdala and allocortical regions (3,16,19,20). However, striatal subregions that receive projections from these regions extend well beyond the traditional boundaries of the ventral striatum (3,16,19). For instance, the ventrolateral striatum (VLS), a region of the CPu located lateral and extending caudal to the NAc, also receives dopaminergic projections from the VTA and limbic projections from the amygdala and allocortical regions (3,16,19). In addition to receiving similar projections, the NAc and VLS both send projections to the ventral pallidum and ventral tegmental area (4,29). Thus, both the NAc and the VLS have similar anatomical connections.

It seems likely that the NAc and VLS may be involved in similar behavioral functions due to the similarities in their anatomical connections. Indeed, localization studies indicate that the NAc mediates spontaneous and psychomotor stimulant-induced locomotor activity (1,9,18), and the VLS mediates spontaneous and stimulant-induced oralfacial movements (10,11,17,24). Localization studies also indicate that the NAc is involved in reward produced by brain stimulation, psychomotor stimulants, and opiates (1,27,28). However, the role of the VLS in reward remains unclear despite previous research. For instance, Carr and White (5) reported a lack of

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CPP following infusion of amphetamine into the VLS. However, their results did not preclude a role for the VLS in amphetamine reward because only one dose was examined. In addition, Kelley and Delfs (15) reported that intra-VLS infusions of amphetamine produced a nonsignificant increase in responding for a conditioned reinforcer. The lack of a significant increase in responding, however, may be due to the intense oral stereotypies that can interfere with operant responding. Lastly, Cousins et al. (8) demonstrated that lesions of the VLS decrease responding for food. However, lesions of the VLS also decrease the rate of food consumption and produce pronounced deficits in food handling (8). Thus, the decrease in responding for food may be due to deficits in food handling, changes in the animals'motivation for food, or both. Because of the concomitant changes in motor behavior in these studies, the role of the VLS in reward remains unclear.

The purpose of the present study was to further examine the role of the VLS in reward and motor behaviors by examining the effects of multiple intra-VLS infusions of amphetamine across a range of doses on locomotion, stereotypies, and conditioned place preference (CPP). CPP assesses drug reward indirectly by measuring the incentive motivational properties of environmental stimuli that have become associated with the drug through classical conditioning. If the drug produces rewarding effects, drug-associated stimuli may acquire positive incentive motivational properties reflected as an increase in the amount of time animals spend in a drug-associated environment relative to a neutral environment. An advantage of this paradigm is that animals can be tested in a nondrugged state, and therefore, impaired motor function during testing is circumvented.

METHOD

Animals

Male Sprague–Dawley rats, weighing 300 ± 10 g at the start of the experiment, were housed individually and maintained on a 12 L:12 D cycle. They were handled for at least 4 days prior to surgery. Rats were anesthetized using sodium pentobarbital (50 mg/kg, IP) in combination with atropine sulfate (10 mg/kg, IP). Bilateral guide cannulae were then implanted as described by Baker et al. (2) into the VLS using the following coordinates derived from the Paxinos and Watson (23) atlas: +0.2 mm AP and ±4.0 mm ML with respect to bregma, and −6.0 mm DV from the surface of the skull. Stylets were placed into the guide cannulae to prevent occlusion of the cannulae. The rats were given at least 4 recovery days prior to conditioning.

Apparatus

The CPP apparatus consisted of rectangular Plexiglas chambers divided into two 36 × 24 × 30 cm compartments. One compartment had pine-scented bedding beneath a wire mesh floor, and all but the front wall were white. The other compartment had cedar-scented bedding beneath a bar grid floor and all but the front wall were black. The front wall of the apparatus was transparent to allow direct observation of the animals' behavior. The wall dividing the two compartments, perpendicular to the front wall, was a removable partition. Previous experiments from our laboratory have demonstrated that untrained rats show equal preference for the two compartments (2,21,22). Each compartment had two sets of photodetectors and light sources mounted to the front and back walls such that the emitted beams were 25 cm apart and 4 cm above the floor. A computer-automated relay system recorded crosses, which were defined as the number of times the two photobeams were interrupted consecutively by the animals moving from one end of the compartment to the other.

Experimental Procedure

The rats received five conditioning trials, each consisting of 30-min exposures to two distinct compartments of the CPP chamber on consecutive days. On 1 day of the trial, rats received bilateral infusions of 0 (n = 17), 2.5 (n = 10), 5.0 (n = 12), 10.0 (n = 10), or 20.0 (n = 11) μg/0.5 μl/side amphetamine into the VLS. The injection cannulae (30-gauge), connected via PE20 tubing to microsyringes in an infusion pump, were inserted bilaterally to a depth of one mm beyond the guide cannulae. One minute later, the pump was activated and 0.5-μl infusions were delivered over 3 min and 10 s. The injection cannulae were left in place for 1 min following the infusion. The rats were then immediately placed into a compartment for 30 min. On the other day of the trial, animals received a sham intracranial injection using an identical procedure as described above, except that the injection cannulae were disconnected from the infusion pump. This procedure was used instead of a saline infusion to minimize tissue damage from repeated intra-VLS infusions. The particular compartment paired with amphetamine, and the order of placement into the drug-paired vs. alternate compartments were counterbalanced across groups. Stereotypies were measured following the first and last intra-VLS infusion using a time-sampling procedure by an observer unaware of the animals' previous treatment. Sniffing and oral stereotypies were assessed using a time-sampling procedure in which the presence or absence of these behaviors was marked every 10 s throughout the 30-min period. Oral stereotypies included licking, biting, and self-biting. Locomotion was also assessed using the automated photocell system. Locomotion (i.e., crosses) and stereotypies were analyzed using ANOVAs with drug treatment as a between-groups measure and injection day (i.e., 1 vs. 5) as a repeated measure. Significant effects were further analyzed using Fisher LSD tests.

The day following the last conditioning trial, animals were tested for CPP. The solid partition was removed from the ap-

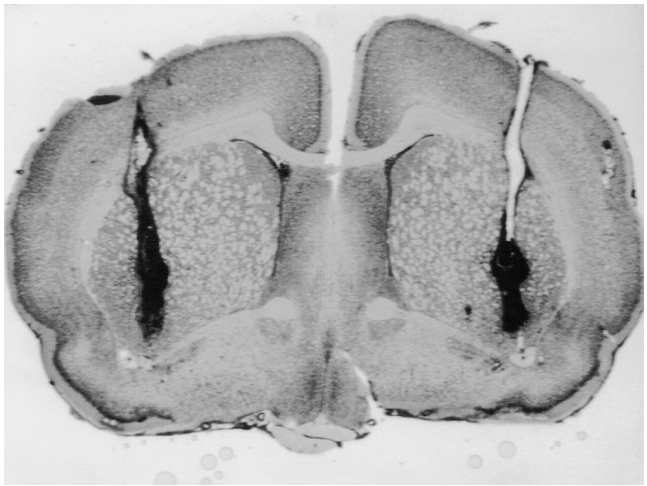

FIG. 1. Representative section illustrating guide cannula tracts and infusion sites in the VLS.

paratus and replaced with a partition containing an opening that allowed the rats free access to both compartments. All rats were placed into the black compartment, such that half began in their drug-paired compartment and half began in their alternate compartment. The amount of time spent in each compartment was then measured for 15 min by an observer who was unaware of the animals' previous treatment. Entry into a compartment was defined as the animals' two front paws touching the floor of that compartment. CPP was defined as a significant increase in the amount of time spent in the drug-paired compartment relative to the alternate compartment. These data were analyzed using nonparametric Wilcoxon signed-rank tests because time spent in each compartment are not orthogonal measures, and therefore, violate assumptions of parametric ANOVA.

The cannula placements were verified postmortem in tissue sections stained with cresyl violet. A representative section illustrating the cannula tracts is shown in Fig. 1. The most ventral point of the tract was designated as the point of infusion and is illustrated for each subject on Fig. 2.

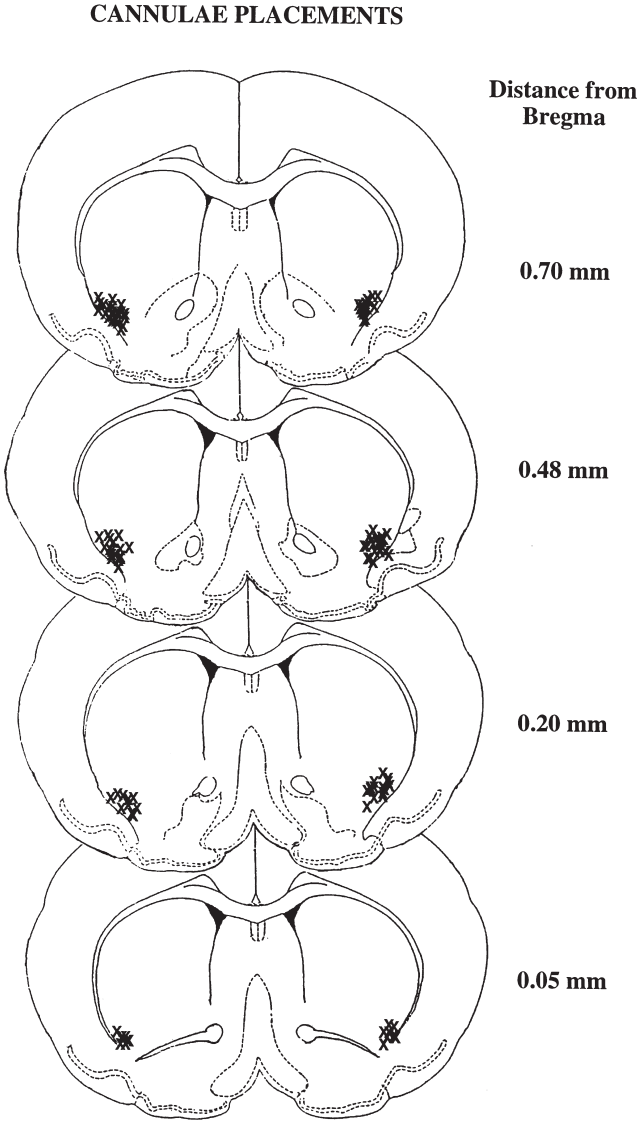

FIG. 2. Approximate position of injection cannula tips, represented by Xs. The drawings were adapted from illustrations in the Paxinos and Watson (23) atlas.

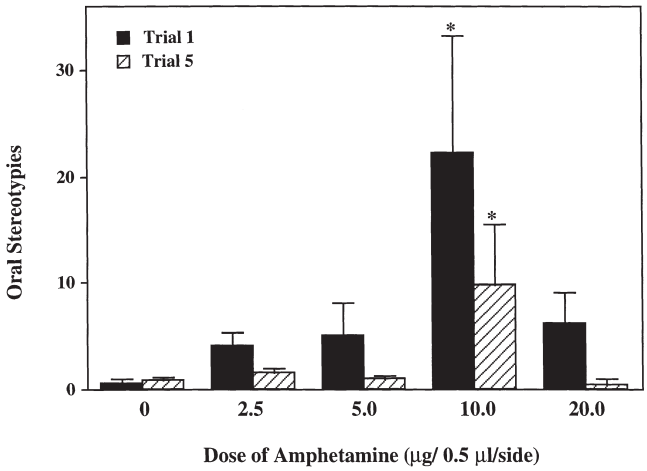

FIG. 3. Time-sampled observations of oral stereotypies (±SEM) totaled across the 30-min test periods following the first and last infusion of varying doses of amphetamine into the VLS. Asterisks (*) represent a significant difference from animals treated with saline alone, $p < 0.05$, Fisher LSD test.

RESULTS

The effects of intra-VLS infusions of amphetamine on the incidence of oral stereotypies are illustrated in Fig. 3. Intra-VLS infusions of amphetamine dose dependently increased oral stereotypies. An overall ANOVA revealed a significant main effect of drug treatment, $F(4, 55) = 3.728$, $p < 0.05$. Intra-VLS infusions of amphetamine produced oral stereotypies

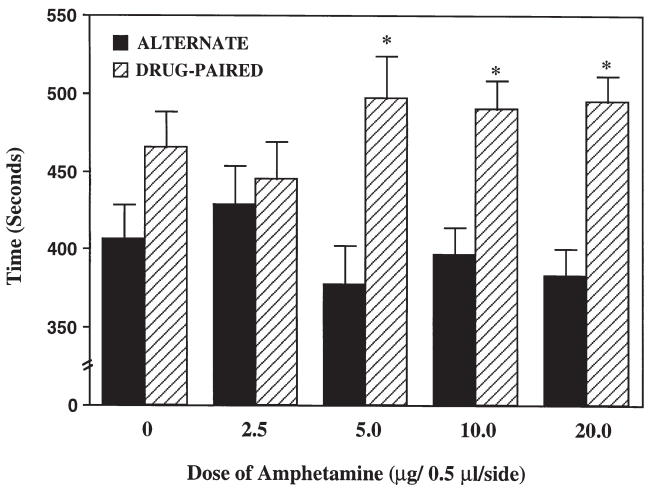

FIG. 4. Time spent (±SEM) in the alternate compartment (black bars) and the drug-paired compartment (hatched bars) during the test for CPP in animals conditioned with varying doses of amphetamine infused into the VLS. Asterisks (*) represent a significant difference in the amount of time spent in the drug-paired compartment relative to the alternate compartment, $p < 0.05$, Wilcoxon signed-rank test.

at each dose; however, only animals treated with the 10.0 μg/side dose exhibited significantly more oral stereotypies than the saline controls ($p < 0.05$, Fisher LSD test). The overall ANOVA also revealed a significant main effect of trial, $F(4, 55) = 7.008$, $p < 0.05$, indicating a decrease in oral stereotypies following the last infusion relative to the first infusion regardless of dosage group. There was no significant interaction between drug treatment and trial.

Infusion of amphetamine into the VLS did not alter sniffing or locomotion (data not shown). The ANOVAs did reveal significant main effects of trial, indicating a decrease in sniffing, $F(4, 55) = 7.32$, $p < 0.05$, and an increase in locomotion, $F(4, 55) = 16.0$, $p < 0.05$, following the last infusion relative to the first infusion, regardless of dosage group. There was no significant interaction between drug treatment and trial.

The effects of intra-VLS infusions of amphetamine on CPP are illustrated in Fig. 4. Intra-VLS infusions of amphetamine dose dependently produced CPP. Animals conditioned with the 0 or 2.5 μg/side doses of amphetamine did not spend significantly more time in the drug-paired compartment relative to the alternate compartment. However, animals conditioned with the 5.0, 10.0, or 20.0 μg/side doses of amphetamine spent significantly more time in the drug-paired compartment relative to the alternate compartment ($p < 0.05$, Wilcoxon signed-rank test).

DISCUSSION

Intra-VLS infusions of amphetamine dose dependently produced CPP. The present results provide the strongest evidence to date that the VLS is involved in the rewarding properties of amphetamine. Although previous studies have not provided strong support, there are several methodological differences between these studies and the present study that may account for the discrepancies. For instance, Carr and White (5) did not detect CPP following intra-VLS infusions of amphetamine. However, in contrast to the present study, the animals were preexposed to the CPP chamber in the absence of amphetamine, which can hinder conditioning due to latent inhibition. Kelley and Delfs (15) reported that intra-VLS infusions of amphetamine produced a nonsignificant increase in responding for a conditioned reinforcer. However, the animals exhibited intense oral stereotypies, which the authors suggest may have interfered with operant responding (15). In contrast, the present study tested animals for CPP in a nondrugged state. Thus, the present study expands upon previous research to suggest that the VLS is involved in amphetamine reward.

As expected, intra-VLS infusion of amphetamine dose dependently produced intense oral stereotypies, and tolerance developed to this effect following repeated administration. The latter finding is consistent with previous research demonstrating that animals develop tolerance to amphetamine-induced oral stereotypies following repeated systemic administration of amphetamine (25). Surprisingly, the magnitude of the oral stereotypy response produced by the 20 μg/side dose of amphetamine was not as robust in the present study as in previous studies (10,17). However, methodological differences likely contributed to the magnitude of oral stereotypies obtained in each study. For instance, animals in the previous studies were tested in their homecages and were allowed to habituate to the testing room prior to behavioral testing. In contrast, the present study did not preexpose the animals to the conditioning apparatus because this may result in latent inhibition that can impede subsequent place conditioning. Thus, the novelty of the testing environment in the present study may have diminished the expression of oral stereotypies. Also, different time-sampling procedures were used to assess behavior, which may vary with respect to sensitivity for detecting dose-dependent changes in the magnitude of oral stereotypies. Nevertheless, the present study expands on previous research by demonstrating that intra-VLS infusions of amphetamine dose dependently produce oral stereotypies, and that tolerance develops to this effect following repeated infusions.

Intra-VLS infusions of amphetamine produced CPP and oral stereotypies without producing an increase in locomotion or sniffing. These findings are consistent with behaviors typically produced following infusion of amphetamine into the VLS, whereas a different pattern of behaviors emerges following infusion of amphetamine into neighboring regions (7,10,12). For instance, infusion of amphetamine or dopamine into the NAc or ventromedial striatum produces an increase in locomotion, sniffing, and rearing (7,10,12). Thus, the pattern of behavioral changes observed in the present study suggests that the effects produced were site specific. Furthermore, the findings are consistent with previous research demonstrating that the range of doses used in the present study produces region-specific CPP. Specifically, doses between 1.25 and 10.0 μg/side produce CPP following infusion into the NAc (5,6), ventral pallidum (13,14), and central, but not the basolateral, nucleus of the amygdala (21).

In conclusion, the traditional view of the function of striatal subregions is that the NAc, but not the CPu, mediates reward. In contrast, the present findings suggest that the VLS, a subregion of the CPu, is also involved in amphetamine reward. Although contrary to current thinking, it is not surprising that the NAc and the VLS are involved in amphetamine reward, considering that both of these striatal subregions have similar anatomical connections (3,4,16,19,29).

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