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Effects of Systemic and Intracranial Amphetamine Injections on Behavior in the Open Field: A Detailed Analysis[1]

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CARR, G. D. AND N. M. WHITE. *Effects of systemic and intracranial amphetamine injections on behavior in the open field: A detailed analysis.* PHARMACOL BIOCHEM BEHAV **27**(1) 113–122, 1987.—Systemic injections of amphetamine result in profound changes in the behavior of animals in an open field. There is an increase in activity, certain species-typical behaviors are produced, and there is a tendency for any elicited behavior to be repeated in a stereotyped way. The present study examined the contributions of dopamine terminal regions to these effects in rats by microinjecting amphetamine directly into one of six discrete sites (medial frontal cortex, nucleus accumbens, anteromedial caudate nucleus, ventrolateral caudate nucleus, amygdala, or the region surrounding the area postrema) and making detailed behavioral observations. This data was compared with the behavior of systemically injected rats that were also observed in the open field. An observer recorded the occurrence of twelve categories of behavior and recorded photocell beam interruptions during five post-injection observation periods. The results confirmed and extended previous accounts of the behavior of systemically injected rats, adding increased snout contact with the environment as an additional effect of amphetamine. Intracranial injections produced changes in activity level from several of the injection sites but there was no increase in the species-typical behaviors associated with stereotypy. Changes in the occurrence of some recorded behaviors were produced by injections into most of the sites and these data are presented in detail.

Amphetamine Open field behavior Locomotion Stereotypy Snout contact Dopamine
Nucleus accumbens Frontal cortex Caudate nucleus Amygdala Area postrema

SYSTEMIC injections of amphetamine result in several changes in the unconditioned behavior of animals. One is an increase in activity level which is apparent even at low doses of the drug, usually reflected in an increase in locomotor activity [20]. A second effect is the emergence of certain behaviors that are particular to a given species and tend to occur at higher doses [29]. The third effect is a tendency for any behavior which is emitted to be repeated. This repetition is apparent in a tendency to repeat the same pattern of locomotion at lower does [31] and at higher doses is reflected in the stereotyped repetition of the species-typical behaviors [20].

In rats placed into an open field, a low dose of amphetamine results in an increase of locomotion and rearing that is accompained by sniffing. The routes that the animal follows tend to be repeated and the places that it stops at tend to be the same [31]. At higher doses, the species-typical behaviors that emerge include sniffing of the floor, nose poking, "bobbing" of the head, padding the floor with the paws, and sometimes licking or gnawing. These species-typical behaviors mesh with the increased level of activity and tendency to repeat behaviors (which are also produced by higher doses), resulting in one or a few of the behaviors being repeated intensely at a high rate. This pattern of behavior is referred to as stereotypy.

Amphetamine exerts its effects primarily by stimulating activity at catecholaminergic synapses. It stimulates the release of dopamine (DA) and noradrenaline (NA) and blocks their deactivation by reuptake [1,15]. Several lines of evidence have suggested that it is primarily amphetamine's stimulation of dopaminergic activity that results in the observed changes in open field activity. These studies have tended to divide amphetamine's effects dichotomously into effects observed at lower vs. higher doses of the drug. Increased locomotion has been associated with lower doses and stereotypy with high doses. Both amphetamine-induced locomotor activity and stereotypy were antagonized by inhibition of DA synthesis [28,35] but inhibition of NA synthesis (from DA) was ineffective [23,32] whereas noradrenergic antagonists were ineffective [20,30]. Selective neurotoxin-

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induced lesions of the brain DA system (intracisternal 6-hydroxydopamine (6-OHDA) with NA protected with desipramine) attenuated both the increased locomotion and stereotypy [17] but selective NA system lesions (6-OHDA, multiple low doses) were ineffective [17]. Stereotypy and locomotion were also abolished by neurotoxin lesions of the DA cell bodies in the substantia nigra but not by neurotoxin lesions of the NA fiber bundles [8].

Having identified the DA system as the critical mediator, attempts have been made to determine which DA terminal areas are responsible for the different behavioral effects. Injections of a DA antagonist (haloperidol) into the nucleus accumbens resulted in an attenuation of locomotion and stereotypy [27]. Stereotypy was also attenuated by injections of DA antagonists into the caudate nucleus [14,27]. Neurotoxin-induced lesions of the nucleus accumbens (6-OHDA) attenuated the increased locomotion but did not affect stereotypy ratings [21]. Lesions of the caudate nucleus (6-OHDA) attenuated "stereotyped behavior maintained in one location" but did not abolish all stereotypy [21]. In a more detailed anatomical study using 6-OHDA lesions, Fink and Smith [13] found data that suggest "mass actions" of the nucleus accumbens, olfactory tubercle and the anteroventral caudate nucleus in producing amphetamine-induced locomotion. Costall and Naylor [7] found that 6-OHDA lesions of either the globus pallidus or central nucleus of the amygdala resulted in an attenuation of stereotypy. Therefore, from the antagonist and lesion studies, it is clear that no one structure has an exclusive role in mediating amphetamine-induced stereotypy or locomotion.

Although a systemic injection of amphetamine has profound effects on open field behavior, the only behavioral change reported for intracranial injections is an increase in locomotor activity obtained from the nucleus accumbens and olfactory tubercle [26]. Although the caudate is frequently assumed to play a prominent role in the stereotypy produced by amphetamine (based on lesion and antagonist studies, above), intra-caudate injections do not produce stereotyped behavior. Stereotypy can be produced by intracaudate amphetamine injections in animals pretreated with a monoamine oxidase inhibitor (Carr and White, unpublished observations), but since this treatment would affect all catecholamine systems, the caudate cannot be singled out.

Since routine observation of rats injected intracranially with amphetamine fails to reveal any obvious changes in open field behavior (except locomotion from the accumbens or olfactory tubercle) the present study made systematic, detailed recordings of open field behavior. It was hoped that these recordings would detect more subtle changes in behavior that would not be detected using the more global observation procedure of standard stereotypy rating scales. Indeed, in a previous study, using a different recording procedure, it was shown that intra-caudate amphetamine injections did produce a slight increase in an aggregate score of the species-typical behaviors associated with stereotypy (e.g., nose poking) [2].

In the present study open field behavior was examined in rats following systemic injection or microinjection of amphetamine into one of six dopaminergic sites. The main forebrain dopamine terminal areas were chosen for examination (medial frontal cortex, nucleus accumbens, anterolateral caudate nucleus, ventrolateral caudate nucleus, and the amygdala) (see [10–12], for a review of dopamine terminal areas). In addition to these sites, the drug was also injected into the region subjacent to the area postrema which includes the nucleus of the solitary tract and the dorsal motor nucleus of the vagus. Both dopaminergic and noradrenergic neurons are present in this region [19,22] and dopamine receptors have been demonstrated in the area postrema itself [33]. Costall *et al.* [6] have lesioned the area postrema and the region subjacent to it and found that the onset of amphetamine-induced stereotypy was facilitated, making it a potentially interesting site for the present study. Following the injections, the subjects' behavior was recorded by an observer using a detailed objective scoring system and photocell beams were used to obtain a measure of general activity.

METHOD

Subjects

Male hooded rats (Charles River, Canada) weighing 300–325 g at the time of surgery were housed individually in suspended metal cages in a room with the lights on between 7 a.m. and 7 p.m. Water and rat chow pellets were continuously available. The subjects in this study had been used previously in research in which they had received intracranial injections of amphetamine and saline in the same dose and volume as used here [3,4]. In addition to the implanted rats, ten unoperated rats were tested with subcutaneous injections for comparison.

Surgery

Stereotaxic surgery was performed to implant stainless steel guide cannulae (0.7 mm outer diameter; Plastic Products Co.). Surgery was performed under 60 mg/kg sodium pentobarbital anesthesia and the implanted cannulae were anchored to the skull using screws and dental cement. The cannulae were aimed at one of six sites in each animal. Coordinates (below) were modifications (based on experience) of the atlas of Pellegrino *et al.* [24] and measured from bregma (anterior-posterior and lateral) with the depth determined by lowering a pre-cut cannula until the plastic sleeve touched the skull. Each rat was implanted bilaterally except for those in the midline area postrema region group which received a single cannula. The brain sites, their abbreviations used here, and the stereotaxic coordinates are:

Medial frontal cortex (MFC). Anterior (A): 4.5, lateral (L): 0.7, rotated 20° laterally from the midline to avoid the superior sagittal sinus and lowered to the depth of a 4 mm cannula (n=19).

Nucleus accumbens (accumbens). A: 3.6, L: 1.5, rotated 20° laterally to avoid the ventricles, and lowered to the depth of an 8 mm cannula (n=17).

Anteromedial caudate nucleus (medial caudate). A: 3.4, L: 1.9, rotated 15° laterally to avoid the ventricles, and lowered to the depth of a 5.5 mm cannula (n=9).

Ventrolateral caudate nucleus (lateral caudate). A: 2.0, L: 4.0, and lowered to the depth of a 7 mm cannula (n=23).

Amygdaloid complex (aimed at the central nucleus) (amygdala). A: 0.0, L: 4.0, lowered to the depth of an 8.5 mm cannula (n=19).

Area postrema nucleus of the solitary tract region (AP region) (aimed for the region just below the area postrema in the surrounding NST). 11.6 posterior to bregma, L: 0.0, and lowered to the depth of a 10 mm cannula, with the posterior edge of the sleeve 2 mm from the skull (note that this placement is 1 mm anterior to where the atlas of Pellegrino *et al.* [24] places the area postrema) (n=13).

Following surgery, a screw-on wire stylet was inserted into the guide cannula. This stylet, and the internal cannulae

used for the injections (0.4 mm outer diameter), were cut so as to extend 0.5 mm from the tip of the guide cannula. An exception to this was the area postrema region placement, which was previously found to be sensitive to mechanical stimulation. This was avoided here by cutting the stylet to be flush with the guide cannula and recessing the internal injection cannula by 0.5 mm. Following surgery, the rats were given one to three injections of penicillin (Derapen) and were given a minimum of one week to recover before the experiment was started.

Intracranial Injections

Injections were made via internal cannulae which were connected to 5 μl Hamilton syringes by polyethylene tubing. For the bilateral injections, two syringes were attached together and two separate lengths of tubing connected them to the left and right internal cannulae. The injections were done simultaneously, infusing the fluid over a one minute period (50 seconds for injection plus 10 seconds for diffusion). Each injection consisted of 10 μg of d-amphetamine sulphate (Smith, Kline and French, Canada) dissolved in 0.5 μl of physiological saline solution, or the saline vehicle alone, injected bilaterally. The 10 μg bilateral dose was chosen as a relatively high dose that has produced clear behavioral effects in other situations (e.g., [3, 4, 26]). This concentration of the drug results in the neural tissue immediately at the cannula tip receiving exposure to a much greater concentration of the drug than it would from the drug dose used for systemic injections (2 mg/kg). The diffusion of the intracranially injected drug into the surrounding tissue would produce a concentration gradient ranging from very high at the cannula tip to virtually zero at some distance from the tip. The steepness of the concentration gradient would depend on the diffusion characteristics of the different types of neural tissue that received the drug. Because we are unaware of how much the drug would diffuse in the different structures used here, we cannot say how much of a given structure (e.g., nucleus accumbens) would have been activated by a concentration of the drug that is comparable to that produced by a subcutaneous injection. We can therefore only make the assumption that some portion of each structure will receive a concentration of the drug that is comparable to the subcutaneous injection and others will receive higher and lower. The clearest indication that this is the case is provided by behavioral data indicating some similar effects from central injections at this dose and systemic injections of 2 mg/kg (e.g. [3]). For the one-cannula AP region injections, the 20 μg was dissolved in distilled water. The concentration of this solution is approximately iso-osmotic with serum and the saline control injections [25]. The AP region injections were done over one minute with thirty additional seconds of diffusion time to compensate for the recessed injection cannula. The subcutaneous group was injected with either 2 mg/kg of amphetamine or the saline vehicle (1 ml/kg).

At the end of the experiment the animals were anesthetized with an overdose of chloral hydrate and perfused intra-cardially with physiological saline, followed by 10% formalin. Their brains were removed and frozen sections were cut at 100 micron intervals for histological examination.

Apparatus

The open field apparatus was a box with three wooden walls and top and a clear Plexiglas front, measuring 45×45×30 cm. The floor was 12 mm wire mesh suspended over a table top. Two sets of parallel photocell beams crossed the floor in each direction, 1.5 cm above the surface, dividing it into nine 15 cm squares.

Open Field Testing

The subjects were placed into the open field for one hour of habituation. On the following day they were placed back into the apparatus for 15 minutes of further habituation. They were then removed and injected as previously described, with either amphetamine or saline (random assignment) and returned to the open field. Behavior was then observed and recorded during five, two minute intervals beginning at 5, 15, 25, 35, and 45 minutes after the injection. During these two minute periods, photocell beam interruptions were tallied by an automatic counter and recorded. An observer also recorded the behavior occurring during these periods using the following procedure. A metronome was set to click every three seconds and the behavior occurring at the click was recorded for thirty consecutive clicks (totalling 90 seconds) during the two minutes. The behavior was noted on preformatted data sheets using two letter codes to represent the following behavioral categories: lying on its belly, standing still, slow locomotion, fast locomotion, grooming, rearing, sniffing with head down (axis close to perpendicular to the floor), sniffing with the head up, gnawing, licking, foot shuffling, and repetitive head movements ("bobbing"). Only one of the above behavioral categories (the most prominent) was scored as occurring at each metronome click. Usually only behavior fitting one category was exhibited at each click, but on occasion, an animal might simultaneously exhibit two. This occurred most often with the category "sniffing up." To facilitate scoring, only the most prominent behavior was scored, according to the following rules.

Lying on its belly. If the animal was on its belly, this category was scored, to the exclusion of any other.

Standing still. If the animal was up on all four legs, essentially motionless, and not actively sniffing, this category was scored.

Slow locomotion. If the animal's main behavior was locomotion at a normal rate, and without noticable sniffing, then this category was scored. Note that the rats sometimes locomote very slowly while sniffing their path. In this case, locomotion is minimal and therefore one of the sniffing categories was scored.

Fast locomotion. This category was differentiated from slow locomotion using the criterion of "locomoting faster than a normal, undrugged rat usually moves in the open field." It required a subjective judgement, but in practice, it was quite easy to judge and usually indicates that the rat was darting about the box.

Grooming. Any sort of grooming received this category score, and grooming rarely co-occurred with any other category.

Rearing. If the rat was rearing up with its weight on its hind paws, then this category was scored. Occasional accompanying sniffing was not scored. If grooming was occurring then "grooming" was scored instead since it reflects a qualitatively different form of behavior.

Sniffing with the head up/down. One of these categories was scored if sniffing was the predominant activity. Both categories required the animal to be sniffing some aspect of the apparatus or the air. Sniffing up versus down was differentiated based on the angle of the rat's head relative to the

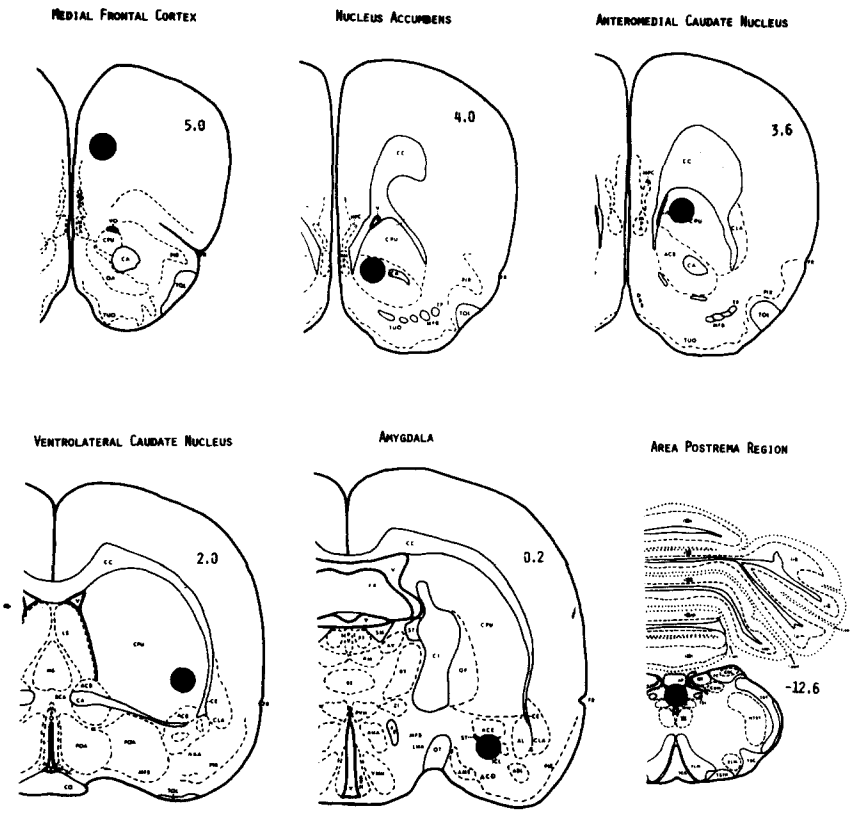

FIG. 1. Representative cannula tip locations for each of the six brain sites. The cross sections are from the atlas of Pellegrino *et al.* [24].

floor. If the axis of the head was perpendicular to the floor, or close to it, then "sniffing down" was scored. Otherwise, "sniffing up" was scored.

Gnawing. This category was scored if the animal was chewing on any part of the apparatus.

Licking. This category was scored if the animal was licking any part of the apparatus.

Foot shuffling. This category was scored if the rat shuffled its paws (alternate movements of two paws) in the absence of forward locomotion.

Repetitive head movements. This was scored if the rat's head followed a repeated pattern of "bobbing" movements. This pattern is not normally observed except in the drugged rat.

In addition to noting the behavior occurring, the observer *also* recorded whether or not the rat's snout was in contact with any environmental surface, which could occur in conjunction with several of the above categories. Snout contact was recorded as an additional measure because Szechtman *et al.* [34] have reported that the dopamine agonist apomorphine greatly increases this component of behavior. It was therefore examined here for amphetamine.

At the end of the fifth observation period, the rat was returned to its home cage. The following day the rats were again tested in the open field using the same procedures (including 15 minutes of habituation) except that each rat's treatment (amphetamine vs. saline) was reversed.

RESULTS

The results of the histological examination are presented as representative placements for each brain site group in Fig. 1. For detailed illustration of each individual cannula placement the reader is referred to Carr and White [3]. Only rats for which both cannulae could be verified as being in the intended site were used in the data analysis.

FACING AND FOLLOWING PAGES

FIG. 2. Effects of amphetamine on twelve behavioral measures of open field behavior. For each behavior, five graphs representing each of the time periods (5, 15, 25, 35, 45 minutes post-injection) are stacked vertically, and the different injection sites are arranged horizontally. The different sites are presented in the same order on each graph and are labelled below the graphs using these three-letter abbreviations; SCU—subcutaneous, MFC—medical frontal cortex, NAC—nucleus accumbens, MCN—medial caudate nucleus, LCN—lateral caudate nucleus, AMY—amygdala, APR—area postrema region. The bar heights correspond to the mean number of times, out of the thirty observations per time period, that the behavior was counted (except for the graph of photocell beam interruptions which represents number of interruptions). The hatched vs. open bars represent each group's response during amphetamine vs. during saline, respectively. The number of animals in each group was SCU—10, MFC—19, NAC—17, MCN—9, LCN—23, AMY—19, APR—13. Note that for "Repetitive Head Movements," this category was only ever scored for the subcutaneous amphetamine group, so only this group is presented on the graph.

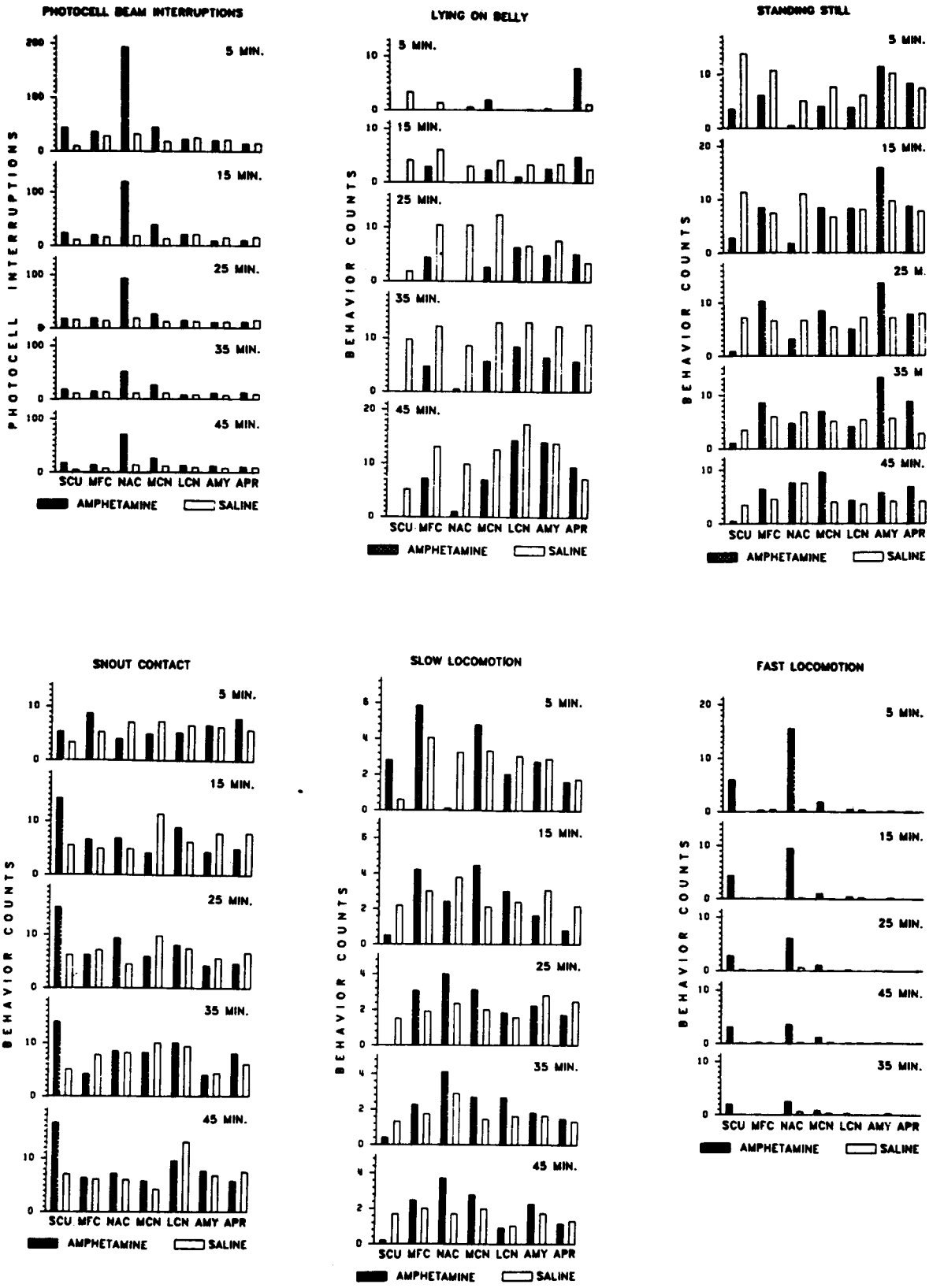
PHOTOCELL BEAM INTERRUPTIONS
PHOTOCELL INTERRUPTIONS
5 MIN.
15 MIN.
25 MIN.
35 MIN.
45 MIN.
SCU MFC NAC MCN LCN AMY APR
AMPHETAMINE
SALINE
LYING ON BELLY
BEHAVIOR COUNTS
STANDING STILL
SNOUT CONTACT
SLOW LOCOMOTION
FAST LOCOMOTION

FIG. 2A

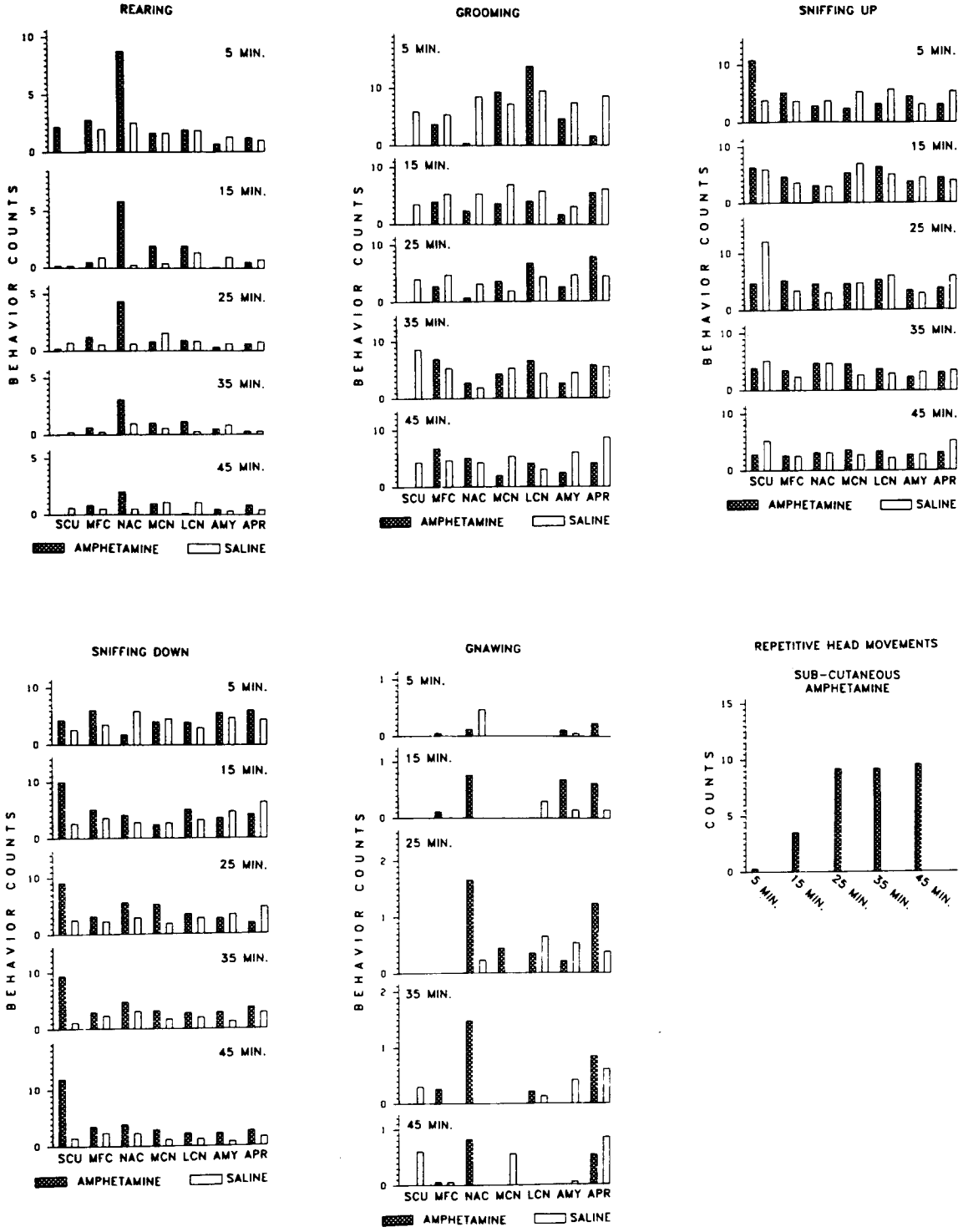
REARING
GROOMING
SNIFFING UP
SNIFFING DOWN
GNAWING
REPETITIVE HEAD MOVEMENTS
SUB-CUTANEOUS AMPHETAMINE
BEHAVIOR COUNTS
COUNTS
5 MIN.
15 MIN.
25 MIN.
35 MIN.
45 MIN.
SCU MFC NAC MCN LCN AMY APR
AMPHETAMINE
SALINE

FIG. 2B

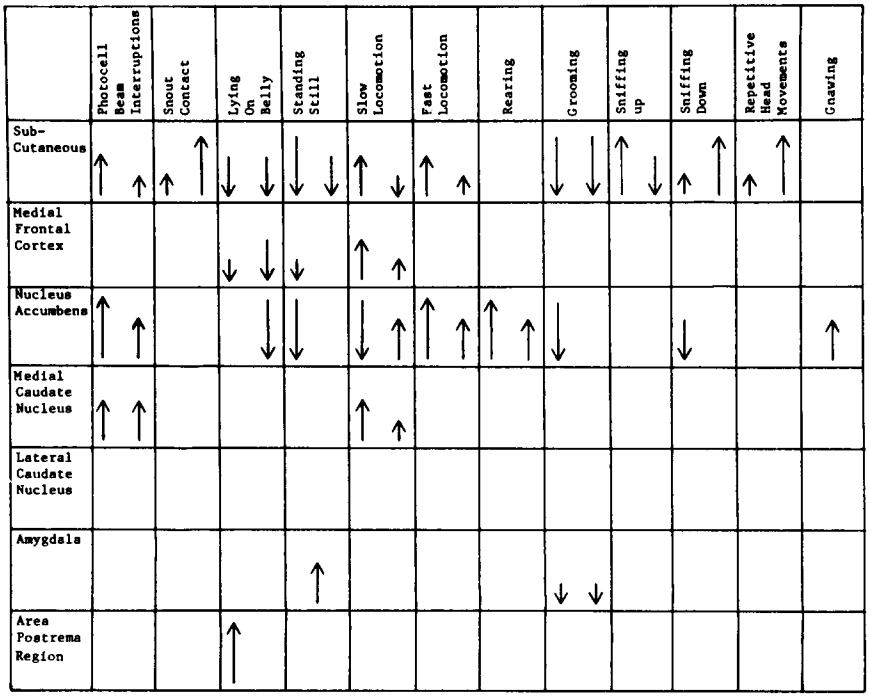

FIG. 3. Summary of the effects of injections of amphetamine into each of the six brain sites and subcutaneously, on each of the behavioral measures. At each site × behavior intersection, the size and direction of the arrow indicate the effects of the amphetamine injection on the behavioral measure. The direction of the arrow indicates whether the amphetamine increased or decreased the occurrence of the behavior relative to saline control injections. The three arrow lengths indicate the relative sizes of the effects as being small, medium or large. The left vs. right placement of the arrows indicates the effects of the amphetamine at earlier vs. later observations during the 45 minute observation period. A centrally placed arrow indicates an effect that occurred mainly towards the middle of the observation period. Only effects that were statistically significant are included here.

The data for the photocell beam interruptions, snout contact and the 12 behavioral categories are presented in detail in Fig. 2 and are summarized in Fig. 3. The data analysis contains a wealth of detail that may appear overwhelming so the reader may wish to examine the data summary in Fig. 3 and then to refer to Fig. 2 and the text below for further detail as desired. Note that the categories foot shuffling and licking were never scored and are therefore not included. Each behavioral measure was analysed (ANOVA BMDP-4VP computer program) using a 3-way analysis of variance (Injection Site × Treatment × Time post-injection) with drug and time as repeated measures. If an interaction was significant, then simple main effects tests from the ANOVA were examined to account for the significant effect. The comparisons of interest were whether, for a given site, the amphetamine injection affected the behavior examined relative to saline, so the simple main effects tests were used as preplanned comparisons, to examine the effects of treatment or treatment × time interactions at a specific site. A summary of the data, presenting the statistically significant effects, is contained in Fig. 3, and the details of the analyses are presented below. To minimize unnecessary information, only the highest level significant interaction(s) from the ANOVA are presented, followed by the results of the simple main effects tests.

Photocell Beam Interruptions

There was a significant site × treatment × time interaction, $F(24,412)=19.89$, $p<0.001$. Treatment by time interactions were significant for the subcutaneous, $F(4,412)=5.75$, $p<0.001$, and accumbens, $F(4,412)=136.75$, $p<0.001$, groups. For the subcutaneous group this reflects significant increases in photcell beam interruptions at 5 ($p<0.001$) and 35 minutes ($p<0.05$) but not at other times ($p<0.1$ in all cases). For the accumbens, the amphetamine-induced increases were significant at all times ($p<0.001$), and the significant interaction reflects the fact that the amphetamine effects were greater at the earlier than at the later times. There was a significant overall increase produced by amphetamine in the medial caudate group, $F(1,103)=10.07$, $p<0.01$, but not for any of the other groups.

Snout Contact

There was a significant site × treatment × time interaction, $F(24,412)=1.58$, $p<0.05$. There was a significant effect of treatment for the subcutaneous group, $F(1,103)=22.34$, $p<0.001$, reflecting increased snout contact for the amphetamine treated rats. For the other groups there were no significant treatment × time interactions or simple effects of treatment ($p>0.1$).

Rearing

There was a significant site × treatment × time interaction, $F(24,412)=2.64$, $p<0.001$. The only significant treatment × time interaction was for the accumbens,

$F(4,412)=12.39$, $p<0.001$, which was reflected in significant increases in rearing at 5–35 ($p<0.001$) and 45 minutes ($p<0.01$) that decreased over time. No other simple treatment effects were present ($p>0.1$ in all cases).

Lying on Belly

There were significant site × time, $F(24,412)=2.31$, $p<0.01$, and treatment × time interactions, $F(4,412)=6.89$, $p<0.001$. Treatment × time interactions were only significant for the accumbens, $F(4,412)=2.63$, $p<0.05$, and the AP region, $F(4,412)=2.74$, $p<0.05$. For the accumbens this reflects less lying on the belly following amphetamine, which was only significant at 25, 35, and 45 minutes post-injection ($p<0.005$). For the AP region, this reflects a significant *increase* in lying on the belly at 5 minutes ($p<0.001$) but no significant effect at any other time ($p>0.05$). For the remaining sites the amphetamine resulted in decreased lying on the belly for the subcutaneous, $F(1,103)=4.10$, $p<0.01$, and MFC, $F(1,103)=7.43$, $p<0.01$, groups but not for any other site ($p>0.05$).

Standing Still

There were significant site × treatment, $F(6,103)=10.38$, $p<0.001$, site × time, $F(24,412)=2.67$, $p<0.001$), and treatment × time, $F(4,412)=6.49$, $p<0.001$, interactions. There were significant treatment × time interactions for the MFC, $F(4,412)=3.05$, $p<0.05$, the accumbens, $F(4,412)=3.18$, $p<0.05$, and the amygdala groups, $F(4,412)=2.62$, $p<0.05$. For the MFC this reflects significant decreases in standing still at 5 minutes ($p<0.05$), but no significant effect at any other times ($p<0.1$). For the accumbens, the interaction is reflected in significantly decreased standing still at 5 ($p<0.05$) and 15 minutes ($p<0.001$) but not at the other times ($p>0.05$). For the amygdala, the interaction is reflected in a significant increase in standing still at 15, 25 ($p<0.005$) and 35 minutes ($p<0.001$) but not at 5 or 45 minutes ($p>0.1$). In addition to the interactions there was also a significant effect of treatment for the subcutaneous group, $F(1,103)=20.17$, $p<0.001$, reflecting an overall decrease in standing still for the amphetamine-treated rats.

Slow Locomotion

There was a significant site × treatment × time interaction, $F(24,412)=2.57$, $p<0.001$. There were significant treatment × time interactions for the subcutaneous, $F(4,412)=3.03$, $p<0.05$, and accumbens groups, $F(4,412)=9.49$, $p<0.001$. For the subcutaneous group, this is reflected in significantly increased slow locomotion at 5 minutes ($p<0.05$) but non-significant decreases at the other times ($p>0.1$). For the accumbens, this is reflected in significantly decreased slow locomotion at 5 minutes ($p<0.001$) but it was significantly increased at 25 ($p<0.05$) and 45 minutes ($p<0.01$). In addition to these interactions, there was also a simple overall effect of treatment for both the MFC, $F(1,103)=6.20$, $p<0.05$, and the medial caudate, $F(1,103)=5.24$, $p<0.05$, groups, reflecting increased slow locomotion in both cases.

Fast Locomotion

There was a significant site × treatment × time interaction, $F(24,412)=18.01$, $p<0.001$. Treatment × time interactions were significant for the subcutaneous, $F(4,412)=7.14$, $p<0.001$ and accumbens groups, $F(4,412)=130.2$, $p<0.001$. No interactions or simple treatment effects were observed for the other groups ($p<0.1$). The interaction for the subcutaneous group is reflected in significantly more fast locomotion at all times (5–35 minutes; $p<0.001$, 45 minutes; $p<0.01$), with the interaction reflecting a greater increase at the earlier observation times. The interaction for the accumbens group reflects the same pattern as above, with significant increases occurring at all times ($p<0.001$).

Grooming

There was a significant site × treatment interaction, $F(6,103)=3.04$, $p<0.01$. Since no interaction involving time was significant, simple effects using the variable are dropped and only overall treatment effects at each site are examined. The subcutaneous group showed a significant amphetamine-induced decrease in grooming, $F(1,103)=12.07$, $p<0.001$. Significant decreases were also seen for the accumbens, $F(1,103)=4.16$, $p<0.05$, and amygdala groups, $F(1,103)=4.70$, $p<0.05$.

Sniffing Up

There was a significant site × treatment × time interaction, $F(24,412)=2.63$, $p<0.001$. There was a significant treatment × time effect for the subcutaneous group, $F(4,412)=10.28$, $p<0.001$, reflected in significantly increased sniffing up at 5 minutes ($p<0.001$) but it was significantly decreased at 25 minutes ($p<0.001$). No other treatment × time or overall treatment effects were observed for the other sites ($p>0.05$).

Sniffing Down

There was a significant site × treatment × time interaction, $F(24,412)=2.09$, $p<0.005$. There were significant treatment × time interactions for the subcutaneous, $F(4,412)=4.31$, $p<0.005$, and accumbens groups, $F(4,412)=5.06$, $p<0.001$, but not for any others ($p>0.05$). The subcutaneous group's interaction was reflected in a significant increase in sniffing down at 15, 25, 35, and 45 minutes ($p<0.001$) but no significant effect at 5 minutes ($p>0.1$). The accumbens group's interaction was reflected in a significant decrease in sniffing down at 5 minutes ($p<0.005$) followed by non-significant increases at the other times ($p>0.05$).

Gnawing

There was a significant site × treatment interaction, $F(6,103)=2.72$, $p<0.05$. The only significant overall treatment effect was for the accumbens, which showed an increase in gnawing, $F(1,103)=16.35$, $p<0.001$.

Repetitive Head Movements

There was a significant site × treatment × time interaction, $F(24,412)=8.19$, $p<0.001$. This behavioral category was only ever scored for the subcutaneously injected amphetamine group. There was a significant treatment × time interaction, $F(4,412)=54.07$, $p<0.001$, which was reflected in significant increases in the occurrence of the behavior at all time ($p<0.001$), most prominantly at 25, 35, and 45 minutes.

DISCUSSION

Subcutaneous amphetamine injections resulted in the commonly reported effects on open field behavior. There

was an initial increase in locomotor activity, recorded as increases in both fast and slow locomotion in addition to increased photocell beam interruptions. Reciprocal decreases in standing still and lying on the belly were also apparent. This was followed by the repetitive, stereotyped behavior as indicated by the repetitive head movements and increased sniffing down. Notably, the activity level was still high, as indicated by increased photocell counts and an absence of lying on the belly and standing still. Grooming was also absent throughout the observation period. The amphetamine produced no increase in gnawing. Sniffing up was increased at five minutes, but was replaced by increased sniffing down at the later times. It therefore appears that sniffing is increased uniformly across time, but that it shifts in orientation from up to down. A finding not previously reported for systemic amphetamine injections was an increase in snout contact with the apparatus that was present througout the observation period. This extends the findings of Szechtman *et al.* [34] who previously reported that increased snout contact was produced by the dopamine receptor agonist, apomorphine.

For the intracranial injections, the most notable finding is the relative lack of effect on open field behavior. The only robust effect that was similar to the effect of a systemic injection was an increase in activity produced by intra-accumbens injections, as has been previously reported [26]. The other behaviors that are related to stereotypy, such as repetitive head movements, increased sniffing down, and snout contact, were not produced from any of the intracranial sites. This is particularly noteworthy because the detailed recording procedure used would have detected even relatively small overall increases in these behaviors, yet none were observed. These negative findings therefore present the question of why the systemic and intracranial routes of injection yield such different results. The demonstration of increased locomotor activity from the intra-accumbens injection suggests that the injection procedures and dose are capable of stimulating the dopaminergic synapses with comparable results to systemic injections. Why then are injections into the other sites unable to produce any evidence of the stereotyped behavioral changes that result from systemic injections? There is no basis for presenting a firm answer to this question but the data suggest that systemic amphetamine injections produce these behavioral changes through its action on some other neural substrate or perhaps by simultaneous action on more than one dopaminergic site. The attenuation of amphetamine-induced stereotypy seen after lesion [21] or neuroleptic-induced receptor blockade of the caudate [27] is therefore interpreted as indicating that while the structure plays a role in the production of stereotypy, other structures must also be involved. The data presented in the introduction (e.g., [7, 21, 27]) supports this suggestion.

In a previous study [2] it was reported that an injection of amphetamine into the dorsolateral caudate produced a slight increase in the frequency of an aggregate of behaviors associated with stereotypy. In comparison with the present study, this positive finding may have been due to the different injection site or to the use of an aggregate rating system instead of scoring behaviors individually. However both studies are consistent in finding none of the easily observable stereotyped behavioral changes that are produced by systemic injections.

Although behavioral changes similar to stereotypy were not observed, the intracranial injection did produce some significant changes in open field behavior. The effects for the different injection sites are summarized here.

The medial frontal cortex amphetamine injections produced only a slight increase in activity reflected in a slight increase in slow locomotion with decreased standing still and lying on the belly.

The nucleus accumbens was the only site to produce the type of dramatic effects on behavior that were seen following peripheral injections. Initially there was a sharp increase in fast locomotion and rearing. These both continued to be significantly elevated, but increased slow locomotion became more prominent. The increased activity throughout the observation period was also reflected in the increased photocell counts and in the initial reciprocal decrease in standing still and later decrease in lying on the belly. As with the subcutaneous group, there was an absence of grooming, although it returned during the later time periods. There was also a slight increase in gnawing; although the absolute amount was very low, it was clearly significant.

The medial caudate group showed a slight increase in activity, as reflected in increased photocell beam interruptions and increased slow locomotion. The possibility that some drug diffusion to the accumbens accounted for this cannot be ruled out.

Except for a slight decrease in grooming, the only significant effect of intra-amygdala amphetamine was a clear increase in standing still. This is notable in that electrical stimulation of the amygdala can evoke "freezing" [18] and the standing still may be related to it.

For the area postrema region, the only effect observed was an increase in lying on the belly, which was prominent at 5 minutes, but was absent after that. A similar, but more prolonged effect is observed after injection of the toxin lithium chloride (unpublished observations). Since lithium injections and intra-AP region amphetamine both result in conditioned taste aversions [3,16], perhaps the lying on the belly is related to an aversive effect of the injection. It is notable that Costall *et al.* [6] found that a lesion of this region resulted in a facilitation of the onset of amphetamine-induced stereotypy. It may be that the lesion eliminated a tendency of a systemically injected animal to lay down, thereby freeing it to engage in stereotypy more easily than if it had an intact AP region.

Two limitations of the present study are first, that the subjects had received intracranial amphetamine and saline injections prior to this study and second, that only one dose of amphetamine was used. Regarding the prior injections, it is possible that these may have slightly affected the magnitude of any behavioral effects due to tolerance or sensitization to the drug, as has been previously reported for repeated systemic injections (e.g. [9]). It is therefore suggested that the precise magnitude of any effects observed in this study may be slightly different than might have been observed with naive subjects. However, there is no evidence to suggest that the direction of the behavioral effects would have been altered by prior injections.

Regarding the use of one drug dose, a dose response study would provide more definitive evidence of any observed behavioral effect. However, the dose chosen was quite high, so if any behavioral effect could be produced, it would likely have been apparent to some degree at this dose. Since the findings are most notable for a lack of observed changes in behavior, a dose response study would likely add little to our knowledge so was not conducted.

In summary, the present study has provided a detailed analysis of the effects of systemic and intracranial am-

phetamine injections on open field behavior. The only component of the open field behavior changes that was caused by subcutaneous injection and was also produced by the intracranial injections was an increase in activity level. It was produced most strongly from the accumbens in the form of locomotion and rearing and to a lesser extent from the MFC and medial caudate as locomotion. No evidence of the species-typical behaviors associated with stereotypy were produced by any of the injections, suggesting that perhaps the syndrome requires simultaneous activation of more than one region.

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