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Implications for Multiple Transmitter Mediation of Amphetamine-Induced Stereotypies

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CHEAL, M. L., M. E. KURKULOS AND L. SILVA. *Implications for multiple transmitter mediation of amphetamine-induced stereotypies*. PHARMAC. BIOCHEM. BEHAV. 17(6) 1305–1308, 1982.—Gerbils were pretreated with the dopamine (DA) receptor blocker, pimozide, prior to stereotypy-inducing injections of d-amphetamine. Some of the stereotypies induced with amphetamine were blocked, but some were not, supporting the hypothesis that multiple neurotransmitters are involved in the mediation of amphetamine-induced stereotypy. In addition, when apomorphine hydrochloride was injected, different stereotypic motor behaviors were induced than were induced with amphetamine. The behavioral changes following amphetamine treatment could be classified into four groups: (1) those that are probably DA related, based on the fact that they were induced with either amphetamine or apomorphine, and amphetamine induction was blocked with pimozide; (2) those that are probably not DA related because they were not induced by apomorphine, and amphetamine induction was not blocked by pimozide; (3) those behaviors that may be incompatible with stereotypic behaviors, were reduced with either amphetamine or apomorphine, but were not maintained with pimozide; and (4) circling, which was induced with amphetamine, blocked with pimozide, but not induced with apomorphine.

Stereotypy Gerbil Behavior Social behavior Amphetamine Apomorphine Pimozide Dopamine Neurotransmitters

D-AMPHETAMINE and apomorphine induce stereotypic behaviors in a large variety of mammals [12,19]. All behaviors in these animals do not become stereotypic following treatment. Some motor patterns decrease, or are eliminated, while others are increased and become repetitious, resulting in a limited repertoire of motor patterns [20]. The behavioral patterns induced with amphetamine are dependent on the time course of drug action [7, 13, 19], the environment [11, 21, 22], previous experience [10,22], housing conditions [23], the species studied [7,19], and the individual animal [7,22]. When animals were placed in a simple, uncluttered, nonstructured environment, d-amphetamine induced different stereotypies in gerbils than in mice, and individual gerbils emitted different behaviors [7]. These differences were independent of either the dose of amphetamine or the time course following drug injection. The simple environment was chosen in order to promote an expression of spontaneous behaviors rather than eliciting specific responses by environment [11] or training [10]. It was suggested that the emergence of different motor patterns following amphetamine treatment could be due to the involvement of multiple neurotransmitter systems [7].

It is unlikely that all behavioral changes induced with amphetamine have the same mechanism, because amphetamine not only increases the release and blocks the reuptake of dopamine (DA) and norepinephrine (NE), but it also interacts with at least three other neurotransmitters: serotonin, acetylcholine, and GABA [8]. To study the influence of multiple transmitters on naturally emitted, drug-induced behaviors, it is necessary to note the quantitative changes in the qualitatively different behaviors [7,13]. Stereotypy scales that have been used extensively are inadequate because they do not differentiate between different behavior patterns. In our method we quantify each behavior pattern shown both prior to drug treatment and at several intervals after injection. Thus, we know quantitatively the behaviors that are induced and those that are attenuated by drug treatment.

In this paper support for the hypothesis that multiple transmitters are involved in the production of stereotypies by amphetamine was obtained by (1) pretreatment of gerbils with the DA receptor blocker, pimozide [1,9], prior to amphetamine injections to determine which stereotypic behavioral units would be blocked; and (2) administration of the DA receptor stimulant, apomorphine, to compare stereotypies induced by amphetamine and apomorphine. It was predicted that pimozide, administered before amphetamine, would block the sterotypic behaviors that were induced by both amphetamine and apomorphine, but that amphetamine-induced behaviors that were not seen following apomorphine injections would not be blocked.

METHOD

Mongolian gerbils (*Meriones unguiculatus*), reared in the laboratory from stock purchased from Tumblebrook Farms

(West Brookfield, MA), were maintained in like-sex groups in 31×61×32 cm glass cages. The gerbils, 13–18 weeks of age (50–68 g), were tested twice, a week or more apart, once in the experimental condition and once in the control condition. Pairs of cagemates were observed in a plain, glass-walled apparatus (44×24 cm) described previously [7]. Strategic placement of two mirrors allowed filming four 4-min observations of the gerbils (4 frames per second on Super 8 film). The film was later projected and observed frame by frame to determine the particular units of behavior that occurred. Two independent observers were used, neither of whom knew the drug condition of the gerbils. One observer made notes during and between filming observations and the other observer exactingly analyzed the film frame by frame to quantify each behavioral pattern displayed. Duration of behavioral units were estimated based on 1/4 sec per frame. The camera was calibrated as reported previously [14]. The behavioral units recorded included: Grooming (normal cleaning of the gerbil's own body), Social Behavior (normal approaching, sniffing, and sitting in contact with the other gerbil), Rearing (standing on the hind legs), Face Washing (a repetitive grooming of the face associated with excessive salivation), Sprawling (lying limply on the abdomen with the limbs extended), Stereotypic Route (moving in a repeated path around the apparatus), Social Stereotypy (moving briefly towards the partner), Circling (turning on the body axis), and Sniffing (continuous sniffing of the floor, characterized by jerky lateral or back-and-forth movements of the head; could be associated with Stereotypic Route and other behaviors). For detailed descriptions of these behavioral units, see Cheal *et al.* [7].

Six gerbils were injected SC with 1 mg/kg pimozide (dissolved in a drop of acetic acid and diluted with saline), or vehicle control, and returned to the home cage. The dose chosen was based on previous experiments in which 1 mg/kg pimozide blocked behaviors induced with apomorphine (1–10 mg/kg) in the gerbil while producing only slight sedation when given alone [4]. Two and a half hours later, pairs of cagemates were placed in the apparatus for adaptation. After 30 min, a 4-min observation was filmed and then the gerbils were injected SC with 4 mg/kg d-amphetamine base (Smith Kline and French). This dose was chosen based on our previous observations of stereotypy after injection of 1–6 mg/kg amphetamine [3, 5, 7]. Repeated observations were made at 30, 60, and 90 min postinjection. These were at the time periods at which amphetamine-induced stereotypic behavior was maximal previously [7]. One week later the experiment was repeated with the gerbils that received pimozide earlier now receiving placebo, and the gerbils that received placebo now receiving pimozide injection. Within animal comparisons were made because amphetamine induces different behaviors in different animals of the same species [7,22].

Six additional gerbils were injected following preinjection observation. One of each pair was injected SC with 3 mg/kg apomorphine hydrochloride (Merck) and the other with saline control. In extensive research with gerbils given a wide range of doses of apomorphine [4, 5, 6], stereotypy was consistently observed following this dose. Notes made on observed behaviors during these experiments suggested a response magnitude that was comparable in intensity to the stereotypy induced with 4 mg/kg amphetamine. Repeated observations were filmed at 15, 30, and 60 min postinjection, based on our earlier observations. Nine to 20 days later, the experiment was repeated with each gerbil receiving the alternate injection.

Differences between conditions were analyzed by the appropriate t-test; Student t for between group comparisons, and matched t-test for within subject comparisons.

RESULTS

Consistent with previous work in gerbils given amphetamine without pimozide [7], Face Washing and Sprawling were observable at 30 min, decreased over time, but were still apparent 90 min postinjection. Stereotypic Route and Social Stereotypy were observed at 30 min and increased at the 60 and 90 min observations. Circling was most frequent at the 60 min observation. Apomorphine induced continual Sniffing (not seen after amphetamine), Stereotypic Route, and Social Stereotypy. Each behavior peaked at 15 min, decreased at 30 min, and returned towards normal at 60 min postinjection.

The behaviors that were changed by the treatments have been grouped in Fig. 1 according to the effect of the drugs. Locomotor activity (measured by projecting the film onto a grid and counting the number of times the gerbils' head crossed the lines) was increased by amphetamine and apomorphine ($p<0.05$), and the amphetamine-induced increase was prevented by pimozide ($p<0.05$; Fig. 1A). Social Stereotypy and Stereotypic Route do not occur in nondrugged gerbils [7] or in those given only pimozide. Amphetamine and apormorphine induced these behaviors ($p<0.05$), and pimozide given before amphetamine prevented them ($p<0.05$).

Normal social behavior, seen in control and pimozide-treated gerbils, was completely eliminated by amphetamine and not maintained by giving pimozide with amphetamine ($p<0.05$; Fig. 1B). Apomorphine reduced the amount of social behavior but did not completely eliminate it. In fact, one male gerbil spent 14 sec mounting his male cagemate during the 4-min observation taken 15 min postinjection, and all of the gerbils initiated at least one encounter during the 4-min observations taken 15 or 30 min postinjection. Face Washing and Sprawling were rarely seen in control, pimozide, or apomorphine-injected gerbils. Amphetamine induced Face Washing and Sprawling, and these were not blocked by pimozide. Although Sprawling could be observed, it is not possible to make quantitative measurement by film analysis [7], so it is not included in the figure.

Rearing and Grooming are normal behaviors seen in control and pimozide-treated gerbils (Fig. 1C). Amphetamine and apomorphine decreased these behaviors ($p<0.05$), but pimozide did not maintain the behaviors at normal levels in amphetamine-injected gerbils.

Sniffing only occurred in apomorphine-treated gerbils (Fig. 1D). Circling was not seen in control, pimozide, or apomorphine-injected gerbils. Amphetamine-induced circling ($p<0.01$) was blocked by pimozide ($p<0.05$).

DISCUSSION

Behaviors manifested following amphetamine injections may be separated into four groups: (1) increased locomotor activity, Social Stereotypy, and Stereotypic Route that appear to be DA mediated because they were blocked by pimozide and were induced by apomorphine; (2) decreased Social Behavior, and increased Face Washing and Sprawling that are probably not DA related because they were not blocked by pimozide, and were not induced with apomorphine; (3) Rearing and Grooming, that may be incompatible with Stereotypic behavior, and were decreased with

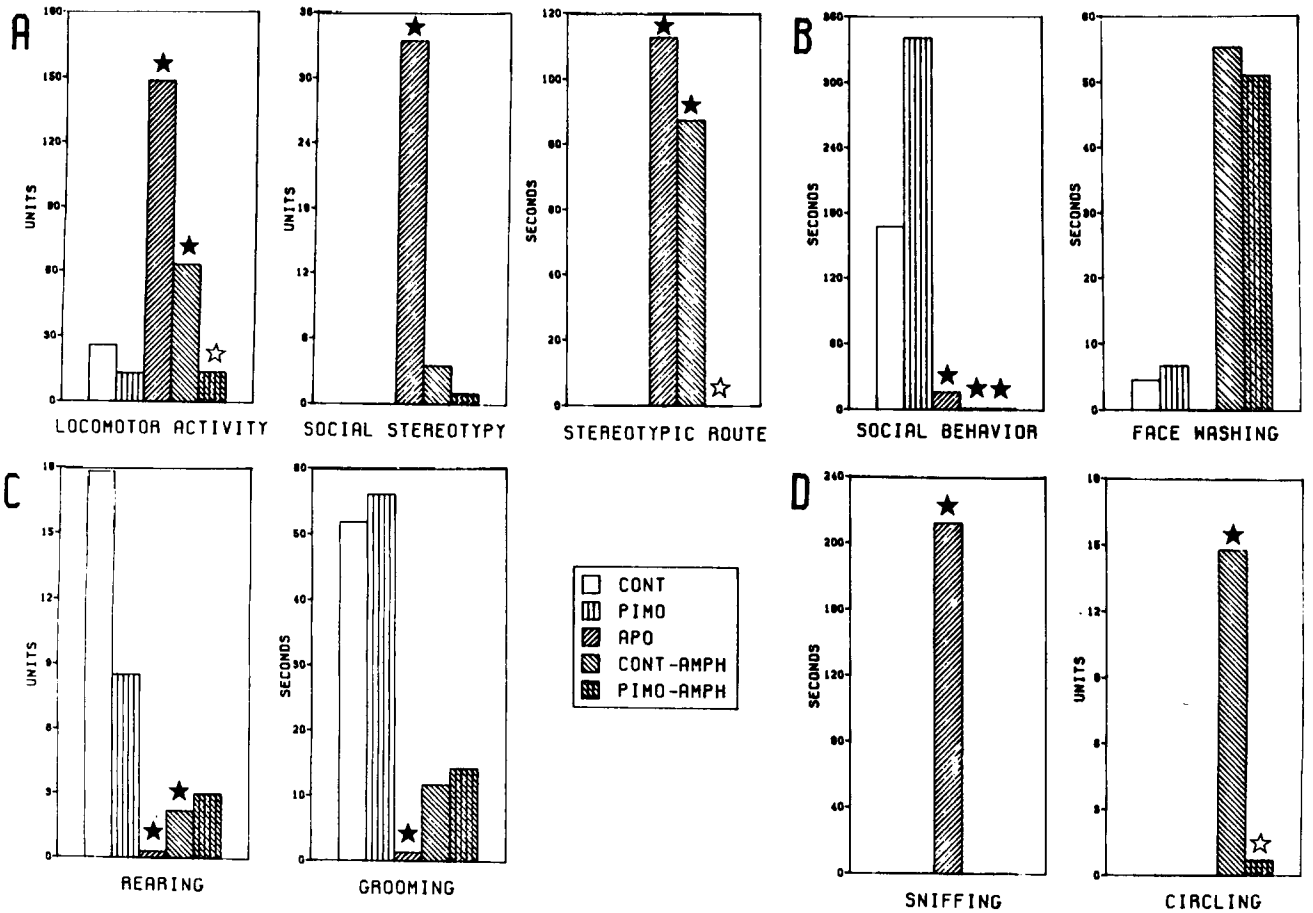

FIG. 1. The mean duration (seconds) or frequency (units) of behaviors exhibited by gerbils during a 4 min observation: CONT, preinjection measures of apomorphine group and vehicle injections (data pooled for graphics, but statistics were computed separately); PIMO, 3 hr after injections of 1 mg/kg pimozide; APO, 15 min after injections of 3 mg/kg apomorphine hydrochloride; CONT-AMPH, 4 hr after vehicle injections and 60 min after injections of 4 mg/kg d-amphetamine base; PIMO-AMPH, 4 hr after injections of 1 mg/kg pimozide and 60 min after injections of 4 mg/kg amphetamine. Although data were collected 30, 60, and 90 min after amphetamine and 15, 30, and 60 min after apomorphine, the times selected for this figure were the times most representative of the behavioral changes. Closed stars, different from CONT, $p<0.05$; open stars, different from CONT-AMPH, $p<0.05$.

apomorphine or amphetamine, but not maintained with pimozide pretreatment; and (4) Circling which is blocked by pimozide but not induced with apomorphine.

The behaviors in C and D, Fig. 1, are of particular interest as they do not fit the model of simple DA or non-DA mediation. Rearing is, of course, incompatible with Face Washing. However, when Rearing occurs following amphetamine it becomes stereotyped and is associated with Stereotypic Route [7]. Amphetamine causes Grooming to be aborted so that the facial aspects are repetitive: the behavior that we have labeled Face Washing. Thus, as Face Washing was not blocked by pimozide, neither would Grooming be maintained by pimozide. Circling may be due to a natural asymmetry of the striatal DA system [15], and postsynaptic stimulation with apomorphine may require a much larger dose (50 mg/kg was used by Jerussi and Glick [15]). Additionally, large doses of apomorphine may be acting on another neurotransmitter system. Pimozide only partially blocked changes in investigatory behavior induced with 10 mg/kg apomorphine [4].

Unfortunately for clear interpretation of the data, neural agents all have multiple actions. Although pimozide has been thought to be a relatively specific DA receptor blocker [1,9], it has also been shown to block a NE-induced increase in cyclic AMP [2]. Similarly, the behaviors induced by amphetamine, but not blocked by pimozide or induced by apomorphine, could occur as a result of amphetamine action on other neurotransmitter systems. As NE [17,18] and serotonin [24,25] have been implicated in mediation of amphetamine-induced behaviors, it would of interest to know whether serotonin or norepinephrine blockers would block some of the amphetamine-induced behaviors in gerbils.

Further questions that need clarification are which DA-mediated behaviors are a result of action on different types of DA-receptors and which are acting in the striatal or limbic DA systems. Although DA-mediated locomotor activity and stereotypy were reported to result from limbic and striatal activity, respectively [16], it is obvious that locomotor activity becomes stereotyped after amphetamine treatment and the distinction between amphetamine-induced locomotion and stereotypies may not be absolute. The methodology used here may be of prime importance in separating behavioral effects of drug treatment. Amphetamine and apomor-

phine were also shown to induce different behaviors in the rat when similar time-course observational methods were used [13].

In conclusion, the hypothesis that multiple neurotransmitters are involved in amphetamine-induced stereotypies was supported because (1) pretreating gerbils with pimozide, the DA receptor blocker, blocked some of the behavioral patterns induced with amphetamine, but not others; and (2) different behaviors were induced by apomorphine than were induced with amphetamine. In spite of interpretational problems due to multiple actions of these drugs, the data reported here provide strong evidence that amphetamine-induced stereotypies are not mediated via a single neural mechanism.

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