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Rapid Decline of Stereotyped Behavior in Rats During Constant One Week Administration of Amphetamine via Implanted ALZET® Osmotic Minipumps

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NIELSEN, E. B. *Rapid decline of stereotyped behavior in rats during constant one week administration of amphetamine via implanted ALZET® osmotic mimipumps.* PHARMAC. BIOCHEM. BEHAV. 15(2) 161–165, 1981.—Groups of rats were subcutaneously implanted with ALZET® 2001 type osmotic minipumps containing d-amphetamine. Three doses of amphetamine (calculated as 0.47, 0.94 and 1.41 mg/kg/hour; measured values were approximately 50% lower), continuously released for 7 days, initially produced locomotor stimulation and stereotyped behavior slightly increasing over the first few days. During the later days, however, locomotor stimulation and stereotyped behavior markedly declined indicating tolerance development. These results contrast the often reported development of sensitization to the behavioral effect of amphetamine observed with repeated injections.

Chronic amphetamine Minipumps Stereotypy Tolerance Sensitization Rat

AMPHETAMINE induced stereotyped behavior in animals has long been associated with schizophrenia-like psychosis in humans, which is often produced during chronic intake of the drug [10, 13, 20, 24]. Animal studies of chronic amphetamine effects have frequently used repeated daily injections of the drug; and a well-documented observation is the gradual increase ("sensitization" or "reverse tolerance") in intensity of some stereotyped behavior, but not all [6], following each injection [3, 9, 19]. It has recently been suggested that this effect parallels the psychotomimetic effects of central stimulants during chronic intake [21]. The sensitization phenomenon in animals has typically been demonstrated in the "free" behavioral situation. In studies involving conditioned behavior, however, the sensitization effect has generally not been observed but, rather, diminution of the drug's effect often develops during repeated injections (for review see [5]). The basis of the sensitization phenomenon is, at the moment, not well understood. Few studies have been concerned with the development of stereotyped behavior under amphetamine treatment in the absence of drug free periods, which might be a prerequisite for the sensitization effect. Previous work with continuous amphetamine administration has made use of implanted silicone reservoirs [7,11]. While these reservoirs have several advantages over multiple injections, they do not have a constant release rate; and, therefore, changes in behavior over time might reflect changes in release rate of the drug. For this reason, the newly developed ALZET® osmotic minipump was of interest in relation to the sensitization phenomenon, since it is reported to release its contents at a constant rate for a lengthy period (7 days) [1].

METHOD

A total of 32 male albino Wistar rats weighing approximately 300 grams (Moellegaards Laboratories, Havdrup, Denmark) were used. Food and water was available ad lib throughout the experiment. The animals were individually housed in wire-mesh cages placed in a quiet room, with lights on from 7:00 to 19:00, under constant 20–21°C temperature. After 10 days in the laboratory, the animals were briefly habituated to the presence of an observer sitting quietly in the room.

Drug Treatment

Pure amphetamine base (ca. 942 mg/ml) was extracted with ether from d-amphetamine sulphate as described by Huberman *et al.* [7] and dissolved in polyethylene glycol 300 (PEG). The animals were randomly assigned to 4 groups (N=8) to receive treatment with ALZET® 2001 osmotic minipumps (ALZA Corporation, Palo Alto, CA) which released calculated doses of 0.47, 0.94 and 1.41 mg/kg/hour of

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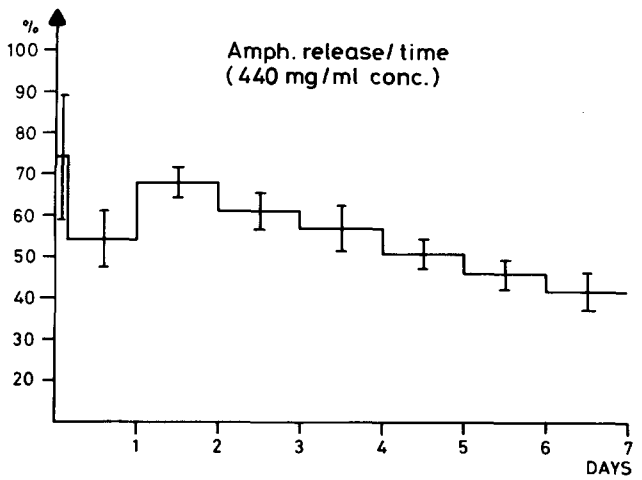

FIG. 1. Average *in vitro* amphetamine release per hour (±SD) during 7 days expressed as a percentage of theoretical (1.02 μl/hour) output rate.

amphetamine base or PEG (control), respectively, based on the *in vitro* release rate of the minipump (1.02 μl/hour) (*in vivo* rate is reported to be 10–15% lower in rats [1]). The concentration of amphetamine base in the minipump was calculated as doselevel (in mg/ml)×bodyweight of the animal. The minipumps were surgically implanted under the skin at the level of the shoulderblades of the animals, under local anesthesia with 0.25 ml of 5% lidocaine.

A generous supply of food pellets was placed directly on the cage floor in order to prevent excessive weight loss during drug treatment.

Behavioral Observations

The animals were coded and placed in a random order in the observation room after implantation. Behavioral observations started the following day. The author, who was blind as to the coding and grouping of animals, rated each animal's behavior after 10 seconds of observation, according to the following scale: 0—inactive or sleep; 1—awake; normal activity such as eating, drinking and grooming; 2—locomotion; 3—discontinuous sniffing over a large area of the cage; 4—continuous sniffing in a restricted area of the cage; 5—continuous licking or biting of the cage grids; 6—awkward crouched posture while continuously self-grooming (biting or licking paws or belly). The animals were scored in a fixed sequence which was repeated 4 times during each of two daily observation periods at 8:30 and 17:00.

In Vitro *Analysis of Minipump Release Rate*

In order to confirm the reported constant release rate of the minipump, as well as to check the uniformity of release rate across the doselevels used, an *in vitro* experiment was designed using the guidelines described by the manufacturer [1]. Three minipumps, which contained the concentration of amphetamine base (438 mg/ml) used for animals receiving 1.41 mg/kg/hour minipumps, were separately submersed in capped test-tubes containing 25 ml of 0.9% saline at 37°C temperature and were transferred to new tubes at 4, 20 and every 24 hours thereafter for 6 days. Three other minipumps, containing 565, 659 and 848 mg/ml of amphetamine base in PEG, respectively, were similarly submersed for 4 hours after filling; data for these samples were not used since the release rate is unstable during this initial period [1]. The minipumps were thereafter transferred to new test-tubes for another 44 hours. The amount of amphetamine released in each test-tube was estimated by the method described by Campbell [4].

Statistical Analysis

The median test [22] was used to test for differences in stereotypy scores over days within groups, and linear regression analysis [22] was applied to release-rate data which was corrected for different submersion periods by conversion to amount of amphetamine released per hour.

RESULTS

The release rate of amphetamine base from the ALZET® osmotic minipump was not quite constant over the 7 days of its effective life (Fig. 1), but declines approximately 3–5% per day, after an initial peak on day 2. Linear regression analysis indicated that this decline was statistically significant (data for the first 4 hours were excluded, as explained above), $t(6)=2.84$, $p<0.025$. The data from the 3 minipumps filled with 565, 659 or 848 mg/ml of amphetamine base indicated that the concentration used in the minipump markedly affects overall release rate which was 0.38, 0.37 and 0.40 mg/hour, respectively; theoretical rates are approximately 0.57, 0.66 and 0.85 mg/hour respectively. Based on these few datapoints, however, the actual doselevel in rats implanted with the 1.41 mg/kg/hour amphetamine minipumps, was therefore approximately 65% lower at this concentration than the theoretical *in vitro* value (1.02 μl/hour), taking into consideration that *in vivo* rates are 10–15% lower than *in vitro* rates [1].

Two animals died in the group implanted with minipumps releasing 0.94 mg/kg/hour of amphetamine base. This was an unexpected finding since none of the animals receiving the highest dose (1.41 mg/kg/hour) died. Data from these animals are excluded and the data from the rest of the group are corrected for this fact.

Normal food and water intake was greatly reduced during the first few days in the animals receiving the higher doses of amphetamine resulting in 15–25% weight loss measured at the end of the drug treatment.

The development of stereotyped behavior during the seven days of continuous amphetamine treatment is shown in Fig. 2. It is seen that dose-dependent stereotyped behavior predominated 24 hours after implantation of the minipumps; and, except for the 0.47 mg/kg/hour group, slightly increased during the following 2–3 days. However, after reaching this peak level, the stereotyped behavior markedly declined and animals with the higher dose levels showed a greater slope of decline. The median test showed a highly significant change of median stereotypy scores over days for the amphetamine treated animals (χ^2 for the 0.47, 0.94 and 1.41 mg/kg/hour group is 63.01, df=6, $p<0.001$, 131.99, df=4, $p<0.001$, and 92.4, df=6, $p<0.001$, respectively), but not for the control group, $\chi^2=11.23$, df=6.

Furthermore, this decline of stereotyped behavior occurred at a much faster rate than can be expected from the slight decline in amphetamine release over days, shown in Fig. 1. As a measure of tolerance development, the daily median stereotypy score for the 1.41 mg/kg/hour group was expressed as a ratio with the respective amphetamine release

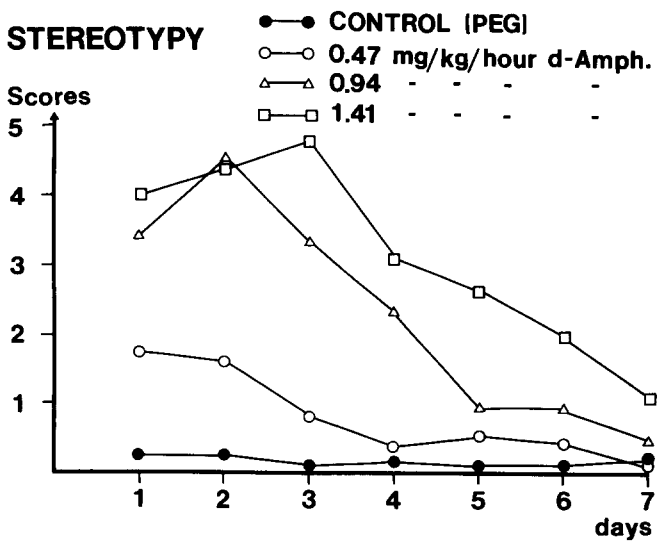

FIG. 2. Average stereotypy scores during 7 days for groups of animals implanted with minipumps releasing PEG (control) or amphetamine at various doses.

per day. A regression line fitted through these points revealed a significant negative slope of -1.14, $t(6)=-2.45$, $p<0.05$. The decline in stereotypy is most pronounced during the later days of the drug treatment and the average stereotypy on the seventh day is close to normal for the drugged animals, although the release of amphetamine only decreased approximately 10% from that of day 1.

However, as shown in Fig. 3, the decline in stereotyped behavior over days is not simply a gradual return to normal behavior, but a marked bimodality of stereotypy ratings develops during the later days. Particularly at the higher dose levels, it appears that during days 3–5 these animals were either in intense stereotypy or showing little or no activity. Inspection of the data from individual animals indicated that this phenomenon was not due to the same animals exhibiting the same behavioral extreme, but different animals would show extreme ratings at different times. However, during days 6–7 the distribution of stereotypy scores is more normal and ratings in the lower end of the scale predominate.

DISCUSSION

The ALZET® osmotic minipump is a useful tool for studying chronic drug effects although its release rate slightly declines over days. However, the overall release rate, as determined in the present experiment, is similar to what has been previously reported by the manufacturer [1]. It is not clear why the concentration of amphetamine base in the pump should affect overall release rate although the present data are only based on few concentrations. This could be due to a number of factors, such as pH-level, viscosity or breakdown of amphetamine, differentially affecting release rate across concentrations.

In the present experiment, the minipump was used to study the course of behavioral changes during the seven days of the effective life of this implant. Chronic amphetamine administration to animals has previously been shown to induce a sensitization to the intensity of stereotyped behavior after each drug injection [3, 6, 9, 19]. Pharmacological evidence indicates that the sensitization phenomenon could be due to changes in the profile of stereotyped behavior, rather than changes in sensitivity to the drug, during the chronic treatment. Depletion of norephinephrine by the chronic amphetamine treatment [23] might affect the influence of dopaminergic (DA) systems, thereby causing a change in the profile of stereotyped behavior [2]. However, if the chronic treatment is continued beyond initial development of sensitization, recent work indicates that the amount of stereotyped behavior declines [16,25], which might be associated with depletion of DA as well. Such effects could underly the initial increase and the subsequent decrease in stereotyped behavior seen in the present experiment. It can further be hypothesized that the change between extreme ratings seen during days 3–5 at the higher doselevels might reflect a fluctuating balance between depletion versus synthesis of DA in the brain.

Alterations in receptor sensitivity often develop during chronic drug treatment, but in this case, the relationship between receptor changes and sensitivity to amphetamine is, however, not fully clear. DA-receptors, thought to be critically involved in stereotyped behavior, are reduced with chronic amphetamine treatment [12]. However, since this effect is only present with relatively high doselevels of the drug, this would not solely explain the decline in stereotyped behavior found at the doselevels tested in the present experiment. Chronic amphetamine treatment has, on the other hand, also been demonstrated to increase DA-receptors, but this effect has so far only been demonstrated upon withdrawal of the chronic treatment, i.e. when measured days after the last drug injection [8,15]. However, the increase in DA-receptors might be involved in the sensitization response, which typically has been observed under conditions of spaced injections.

Behavioral mechanisms have been implicated in the sensitization phenomenon [14] which, however, would not be surprising, given that the drug acts as a powerful reinforcer and has strong "internal" stimulus properties [17,26]. From a behavioral point of view, this would readily mediate conditioned drug effects when repeatedly presented during chronic injection schedules. In the present experiment, the absence of drug free periods and injection related stimulus variables, could be significant in the finding of decline, rather than sensitization, to the stereotyped behavior during the chronic drug treatment.

Finally, the tolerance development reported here, could also be explained by the general model proposed by Schuster *et al.* [18]. These researchers suggested that tolerance develops to the aspects of drug effect interfering with reinforcement density. In this case, the progressive lack of sleep, food and water during the constant performance of stereotyped behavior would increasingly affect the animal, eventually leading to adaptive diminution of stereotyped behavior such as Schuster's model predicts. Furthermore, the bimodality of stereotypy ratings seen during days 3–5 might reflect a shifting balance between the "stimulating" effect of the drug and the severe lack of sleep presumably present at this time.

In conclusion, the present results indicate that continuous administration of amphetamine via implanted osmotic minipumps rapidly leads to a decline in stereotyped behavior, initially present. This effect contrasts with the sensitization typically found with repeated injections of the drug. It is suggested that the sensitization phenomenon might be dependent on both behavioral and pharmacological factors,

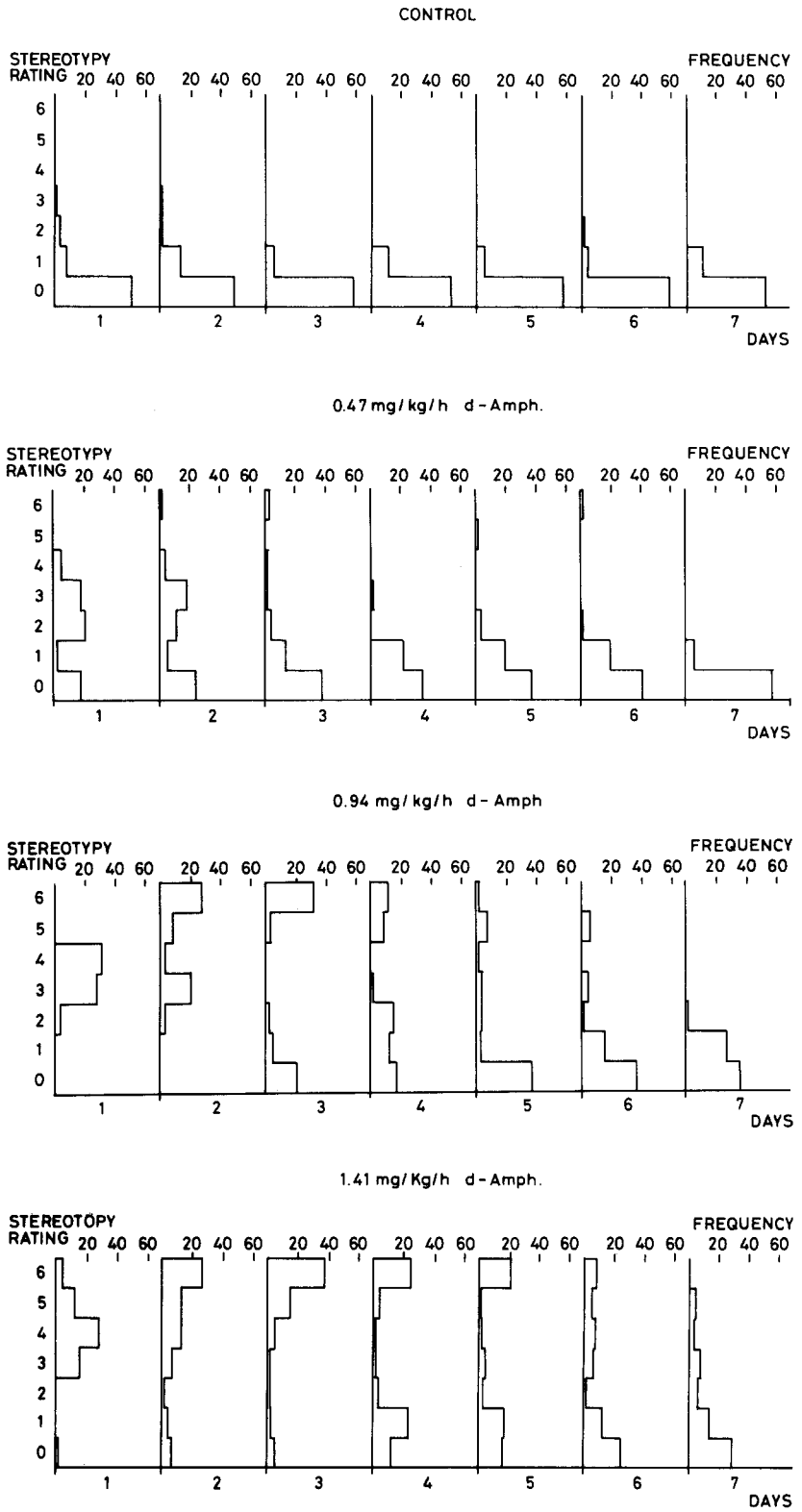

FIG. 3. Distribution of stereotypy ratings in daily frequences for animals implanted with minipumps releasing PEG (control) or amphetamine at different doses. The maximum frequency for each score is 64, based on 8 animals per group, observed 4 times, twice daily.

such as injection related stimulus variables or presence of drug free periods between injections. The present results might have relevance for sensitization processes hypothesized to parallel the psychotomimetic properties of amphetamine in humans [21].

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