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Effects of a High-Dose Treatment of Methamphetamine on Caudate Dopamine and Anorexia in Rats[1]

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BITTNER, S E., G C WAGNER, T. G AIGNER AND L. S. SEIDEN. *Effects of a high-dose treatment of methamphetamine on caudate dopamine and anorexia in rats* PHARMAC BIOCHEM BEHAV. 14(4) 481–486, 1981 —Dose-effect curves of *d*-methamphetamine (MA) on intake of sweetened condensed milk by rats were obtained before and after twice a day treatment for four days with either saline (control) or a high (50 mg/kg) dose of MA previously shown to decrease the dopamine levels of the caudate. The animals that were more sensitive to MA's anorexic effects during the before-treatment determination were found to be more sensitive to the lethal effects of the high-dose treatment. This treatment produced a six month decrease in brain dopamine but no change in the anorexic effect on milk intake or in the stereotypic behavior elicited by the drug. Subsequently, the daily administration of 2.5 mg/kg of MA, 15 min before presentation of the milk, to both control and treatment groups produced tolerance to the drug's anorexic effect. After 4 to 5 weeks of repeated administration of this dose there was a significant difference between the control group's intake of milk and treatment group's intake as well as body weight. These differences indicate an effect of the treatment upon the formation of tolerance to the anorexic effects of MA

Methamphetamine Tolerance Anorexia Dopamine

A LONG term (3–6 month) decrease in the catecholamine (CA) content in the brains of rhesus monkeys was observed after the repeated administration of *d*-methamphetamine (MA) [8]. The greatest decrease in CA levels was for the caudate dopamine (DA) content, with lesser decreases found in other regions of the brain for norepinephrine (NE). These monkeys also manifested a persisting behavioral tolerance on a differential-reinforcement-of-low-rate task, consequent to this MA treatment [2]. Similar neurochemical effects were subsequently found in the rat and guinea pig, utilizing a treatment period of 30 days with doses of 25–50 mg/kg/day of MA for the rats and 6–30 mg/kg/day of MA for the guinea pigs [13]. Depletions of caudate DA content in the rat were 25% to 60% and in the guinea pig 50%. These long-lasting DA depletions were demonstrated in the rat after a shorter (four days) treatment period with doses of MA from 50 to 100 mg/kg/day [12]. The DA depletions were found to last at least 8 weeks [12] while NE depletions were not found in either of these rodent studies. Analogous to the monkeys, rats which had undergone the later treatment several months earlier were found to be more tolerant to the disruptive effects of MA on a fixed-interval task [11]. These studies suggest that the CA depletions induced by the high-dose treatment with MA have long-lasting functional consequences.

The purpose of this study was to investigate in the rat additional consequences of the four day, 100 mg/kg/day treatment with MA. First, dose-effect curves were determined for both the anorexic effects of MA and the effects of MA on stereotypic behavior before and after a four day treatment with either a high dose of MA or the vehicle (control). Next, both MA and control treated rats were administered 2.5 mg/kg of MA on a daily basis and the rate of formation and subsequent loss of tolerance to the anorexic effects of MA were compared between the two groups.

METHOD

Subjects

Twenty-four male Sprague-Dawley (Holtzman Co. Madison, WI) rats weighing from 242–294 g were used. The rats were individually housed in suspended metal cages. The room lights were automatically turned on at 0600 hr and off at 1800 hr. Except where noted, the rats were food-deprived with water available ad lib.

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General Procedure

The procedure for milk drinking was as described elsewhere [14]. For 15 min at the same time each day access was provided to sweetened condensed milk (Bordens Co.) diluted 2:1 with water. The milk was delivered using a standard drinking tube and calibrated bottles (Wahmann, Baltimore, MD). The only additional supplement to the diet of milk was 5 g of standard rat chow (Teklad Mouse and Rat Diet, Winfield, OH) given immediately after the milk. All injections were given intraperitoneally (IP) 15 min before the milk was presented. Doses of MA were calculated as the hydrochloride. The vehicle was 0.9% saline with a concentration adjusted to yield a volume of 0.1 ml/100 g body weight

Before Treatment Dose-Effects on Milk Intake

After the daily milk intake had stabilized, (less than 10% variation during the last 3 day period) the animals were randomly divided into control and treatment groups. Drug was given every fourth day with doses presented in a random order. The animals were then removed from the milk diet and placed on ad lib food and water for 4 days

High-Dose Treatment

Fifty mg/kg of MA was given subcutaneously 2 times a day for 4 days as previously described [12]. The control group received saline injections. At the end of the four days of high-dose treatment 3 rats had been dropped for nonrelated causes and 6 animals had died as a result of the drug treatment. This left 6 control animals and 9 treated animals. All calculations except where noted are based on data from these 15 animals

After Treatment MA Dose-Effects on Milk Intake

For 14 days after the high-dose treatment, both groups were maintained on ad lib food and water On the 15th day, the animals were again food deprived. On the 16th day, presentation of the milk was restarted. After 10 days milk intake for the animals was stable. Determination of the second dose-effect curve was then started on day 27.

Tolerance Development

Following the second dose-effect determination, the animals were placed on food and water ad lib for 15 days. Afterwards, the rats were food-deprived for 2 days On the 3rd day milk was presented. After 10 days milk intake was again stable. On day 11 both the control and treated groups received the first daily administration of 2.5 mg/kg of MA. The MA was given IP 15 min before presentation of the milk. The dose of MA was chosen to decrease the milk intake of the rats to about 20% of baseline levels. After 39 days, when the control group milk intake was at baseline levels, a dose effect curve was determined for both groups by substituting the test dose of MA for the 2.5 mg/kg daily dose. Test doses were given in random order with three days of 2.5 mg/kg of MA intervening for restabilization of milk drinking between probe doses.

Removed from Daily Administration

After 11 days of discontinuation of the 2.5 mg/kg daily administration of MA, the base line level of milk intake was stable. During days 12–21 after removing the animals from the daily administration, final dose-effect functions were determined. Each animal received 3 test doses of MA with 3 days between each test dose to stabilize milk intake.

Stereotypy Rating

An estimate of stereotypic behavior was obtained for each animal during the before and after treatment dose-effect determinations. To minimize observer bias the doses of MA were encoded and randomized. The estimate was made after the sweetened condensed milk had been removed (approximately 30 min after the drug injection). Each animal was observed for 15 sec and rated once with the scale developed by Scheel-Kruger [9]. This scale provides a method of quickly rating the behavior of the animal into one of six categories and it has been shown to be sensitive to the changes in stereotypic behavior with increasing doses of amphetamine [9].

Determination of CA Content

The animals were maintained on ad lib food and water for one month after the last injection of MA (6 months after the high dose treatment), at which time they were killed by decapitation. The caudate was dissected over ice as described elsewhere [13]. Brain parts were wrapped in aluminum foil and frozen in liquid nitrogen until the CA levels could be determined using high performance liquid chromatography and alumina adsorption with subsequent spectroflurometric determination [1, 6, 10].

Statistics

Comparisons of group means were made using the unpaired Student t-test [3] with $p<0.05$ accepted for significance.

RESULTS

Initial Determination of MA Sensitivity

After 19 days of milk presentation, the mean (±S.E.M.) intake of the experimental group was 29.8±2.7 ml and the control group was 32.8±5.6. Mean body weights were 255±13 g for the experimental group and 248±15 g for the control group. There were no significant differences in either of these two measures between the groups

The first dose-effect functions (before the high dose treatment) are shown in Fig. 1A. There were no differences in the sensitivity of the two groups to the anorexigenic effects of MA. However, the animals that did not survive the high dose treatment were significantly more sensitive to MA's disruption of milk drinking.

After High-Dose Treatment

After the high-dose treatment of MA, milk intake for both the control and the surviving treated groups was allowed to restabilize. There were no differences in the mean milk intake between the control and treated groups (37.2±4.1 *vs* 37.8±3.5 ml respectively) There were no significant differences between the body weights of these two groups (383±18 and 363±24 respectively).

When the second dose-effect functions were determined (Fig. 1B), both curves were shifted significantly to the left of the initial determination. However, there were no significant

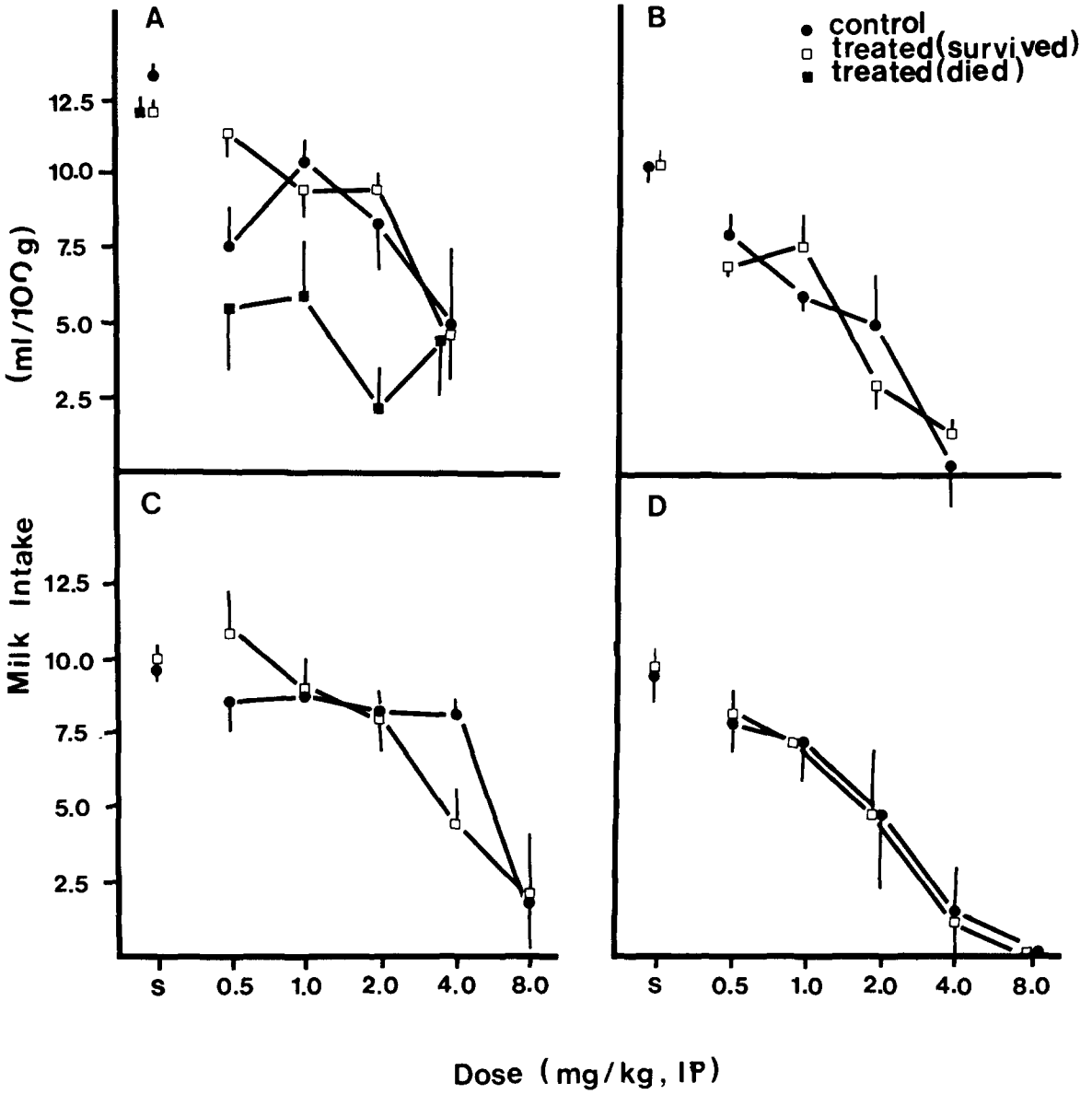

FIG. 1 Effect of various doses of *d*-methamphetamine HCl (MA) upon milk intake in rats. (A) before a high-dose treatment, (B) after a high-dose treatment; (C) while administered daily injections of 2 5 mg/kg of MA; (D) after discontinuing the daily administration of drug Values represent the mean (±S E M.) milk intake in ml/100 g.

differences between the control and the treated group's MA dose-effect curves after the high-dose treatment.

Tolerance Development

Milk intake during the repeated administration of 2.5 mg/kg of MA is shown in Fig. 2A. At the start of the daily administration, both groups showed a comparable decrease in milk intake. As the treatment continued, the control group became tolerant to the disruptive effects of MA upon milk drinking. The treated group also showed a marked degree of tolerance, but continued to show a small drug effect. There was a significant (20%) ($p<0.05$) difference in mean milk intake for days 36–38 (Fig. 2A) between control and treated groups.

Figure 2B shows that body weight covaried with the intake of milk. The high-dose treated animals did not recover as much body weight as did the control animals but at no point was this difference significant. Final (day 38) body weights were 366±48; 400±46 respectively.

Determination of the dose-effect function during the repeated administration started on the 39th day. Both the treated group's and control group's dose-effect curves (Fig. 1C) showed a shift to the right from the after-treatment dose-effect curves.

The dose-effect curves for both the treated and the con-

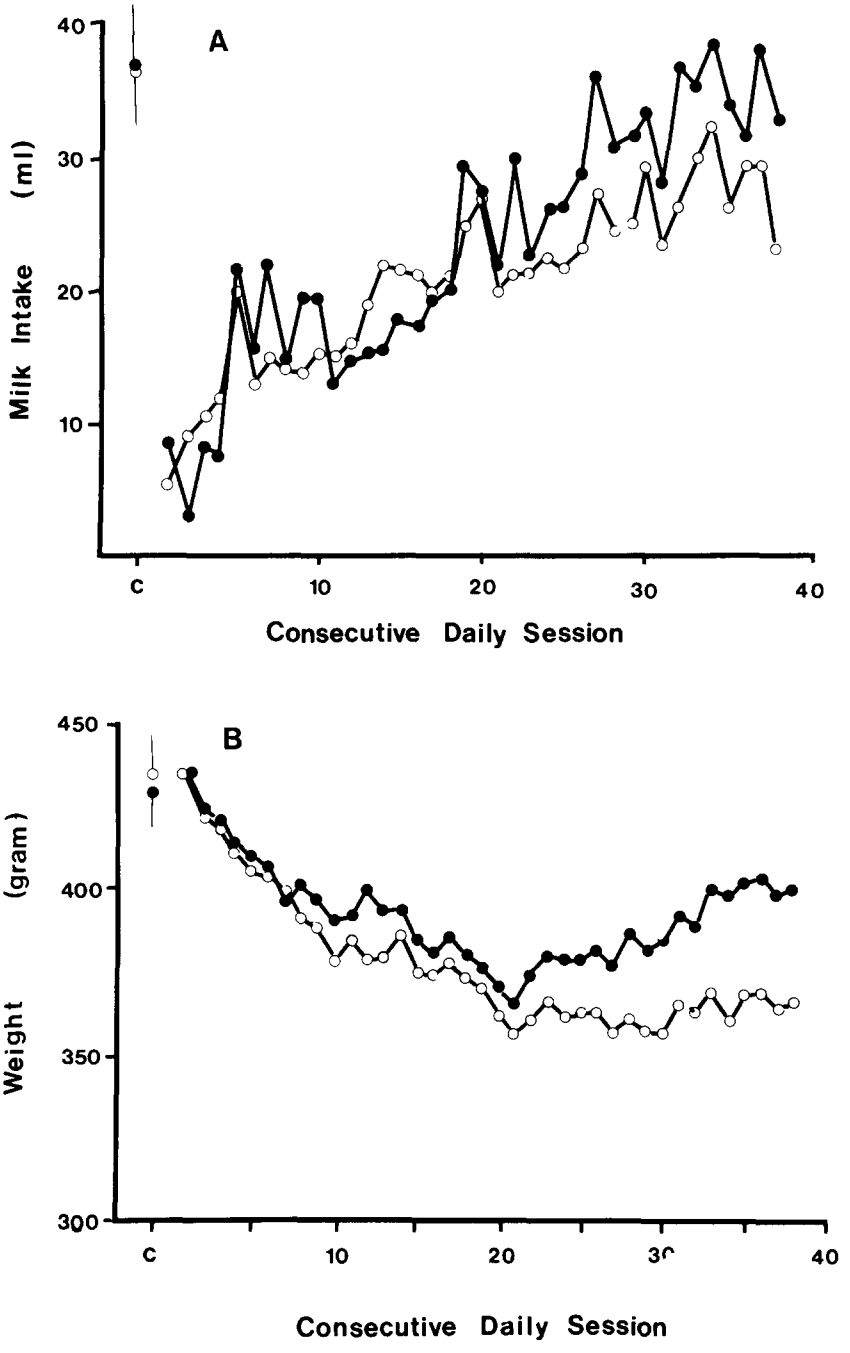

FIG 2. (A) The effect of repeated administration of MA upon the actual mean milk intake in ml of rats treated with a high-dose regimen of MA (○——○) compared to the control group (●——●) (B) The effect of repeated administration of MA upon the mean body weight of rats treated with a high-dose regimen of MA (○——○) and compared to the control group (●——●). See text for significant effects.

TABLE 1

EFFECT OF d-METHAMPHETAMINE TREATMENT ON BRAIN CATECHOLAMINE LEVELS

		Caudate	Rest of Brain	
	N	Dopamine	Dopamine	Norepinephrine
Controls	6	10 42 ± 0.63	0.44 ± 0.02	0 352 ± 0 01
Treated	9	7.29 ± 0 41*	0 35 ± 0.01†	0 362 ± 0 01

Data shown are mean ± S.E.M. in mg/g.
*Significantly lower than control group ($p<0.001$)
†Significantly lower than control group ($p<0.01$).

trol groups after 12–21 days without administration of drug are not statistically different and are shown in Fig. 1D. Both curves shifted back to the nontolerant levels showing both groups have lost their tolerance to MA.

Estimates of Stereotypic Behavior

The proportion of animals in each rating category was a function of the dose of MA. With the increasing doses of MA there was a greater proportion of animals with a higher rating of stereotypic behavior. For doses 1, 2 and 4 mg/kg there was a slight but not significantly increased proportion of animals with a higher rating of stereotypic behavior after the MA treatment than before the treatment. This higher rating was true for both the control group and the treated group. The shift in proportion indicated an increase in sensitivity to MA between the two estimates of stereotypic behavior. There was no difference between the ratings of the treated group and the control group which could be attributed to the high dose regimen of MA (data not shown)

Brain Dopamine and Norepinephrine Levels

Table 1 shows the mean DA and NE brain levels for the two groups. Caudate DA levels were decreased by 30% ($p<0.001$) in MA treated rats when compared to controls, while in the rest of the brain, DA was decreased by 20% ($p<0.01$). There were no significant differences in the NE levels in the rest of the brain for the two groups.

DISCUSSION

Long-term (6 month) decreases in DA and NE levels have been reported in rhesus monkeys following the repeated administration of MA [8]. Depletions of regional brain DA levels were also found in rats after a relatively short (four days), high-dose treatment with MA [12,13]. The decreases of DA levels in the present study extends these effects to 6 months in the rodent and agrees with the changes previously reported [13].

Before the rats were administered the high dose treatment of MA, estimates were made of each animals sensitivity to the anorexic effects of MA on milk intake and of their stereotypic responses to the drug. Those animals showing the greatest sensitivity to the anorexic effects of the drug died during the 4 day high-dose treatment. No such correlation was found for the sensitivity of the animals to the stereotypic effects of the drug. Therefore, the sensitivity of the animals to the anorexic effects of MA may have been a good predictor of lethality during the high-dose treatment.

It has been suggested that the decrease in CA levels found in the rhesus monkeys after repeated daily injections of MA could be related to their permanent tolerance on a DRL task [2,8]. In rats a decrease in sensitivity to the disruptive effects of MA following a high dose MA treatment on an operant behavior has been observed [11]. Also, a decrease in sensitivity to the anorexic effects of MA was observed following specific DA depletions induced by 6-hydroxydopamine (6-OHDA) [4,5]. No such decrease in sensitivity was found in this study for either the anorexic effects of MA or for the drug effects on stereotypic behavior. However, it should be pointed out that the degree of DA depletion was far less in this study (20–30%) than in the above (6-OHDA) study. Finally, the slight shift to the left in the dose-effect curves following the treatment was apparently non-specific since it occurred in both MA and saline treated rats and also was apparent in the measure of stereotypic behavior.

The present study has shown that the high dose regimen increased the rats sensitivity to the disruptive effects of repeatedly administered MA on milk consumption. This relative increase in sensitivity appeared only after 3 weeks of daily injections of MA and was accompanied by a greater loss of weight through the course of the repeated daily administration. It should be emphazied that the decreased milk consumption of treated rats during this period was depicted in Fig. 2 as absolute ml intake. When considered as ml/100 g body weight (as Fig. 1) these differences are attenuated. However, these two groups of rats did have essentially the same body weight at the start of the repeated administration period. Our data are, therefore, consistent with the proposal that DA neurons may be necessary for the occurrence of adaptive changes needed for the formation of tolerance [4]. The loss of tolerance after discontinuing the daily regimen demonstrated the requirement for continuing the repeated administration of the drug for tolerance to exist [7,14].

In summary, the results of this experiment show that a high-dose treatment of MA was more likely to cause lethality in the animals shown to be more sensitive to the drug's anorexic effects. The high dose treatment did not affect the daily milk intake of the rats, the sensitivity of the rats to MA's disruptive effects on milk intake or the stereotypic behavior induced by the drug. The treatment did decrease the ability of the animals to become tolerant to the disruptive effects of MA on milk-drinking behavior. In light of the work on 6-OHDA treated rats [4] it seems probable that this latter effect is related to the drug-induced deplations of dopamine.

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