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Effect of once weekly treatment with 3,4-methylenedioxymethamphetamine on schedule-controlled behavior in rats

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Abstract

The present study examined the effects of 3,4-methylenedioxymethamphetamine (MDMA), before and after once a week dosing, on the behavior of rats responding under a fixed ratio 20 schedule of reinforcement. Acutely, cumulative doses of MDMA dose-dependently decreased responding when compared to a series of water injections. Rats were then separated into two groups, one of which received only weekly MDMA ('paired') while the other received an additional injection of water each week ('unpaired'). Weekly dosing with MDMA resulted in significantly increased responding at low doses in the paired group but not in the unpaired group. When water injections were readministered there was a significant increase in responding in both groups. During the weekly regimen, locomotor activity also increased significantly over time after both water and MDMA injections. In conclusion, it appears that even weekly dosing with a small amount of MDMA can have long-lasting effects that are manifested in both operant and spontaneous behavior and that may be mediated by a conditioning mechanism.

Keywords: MDMA (3,4-methylenedioxymethamphetamine); Conditioning; Schedule-controlled behavior; Locomotor activity

1. Introduction

In the past decade, recreational use of 3,4-methylenedioxymethamphetamine (MDMA), popularly known as 'Ecstasy', has increased greatly, not just in the United States but in Europe as well. MDMA is an amphetamine derivative and thus can be characterized as a psychomotor stimulant. However, unlike other psychomotor stimulants such as cocaine or amphetamine that may be taken on a daily basis, MDMA is used primarily on weekends at dance parties called 'raves'. Because people who use MDMA at 'raves' generally only take the drug on a single night during a weekend, one purpose of this study was to examine the effects of once weekly administration of a 'relevant' dose of MDMA on operant performance in rats. Operant performance has been used extensively to determine the effects of drugs on behavior in laboratory animals and sometimes, in humans. The rate decreasing effects of MDMA on multiple schedule fixed-ratio (FR) and fixed-interval (FI) components in pigeons have been reported (Nadar et al., 1989); and in mice, MDMA decreased rates of responding on a fixed ratio schedule (Glennon et al., 1987).

There is a vast literature on the effects of chronic administration of psychomotor stimulants such as amphetamine, but very little is known about the chronic effects of MDMA. In a study done by Zacny et al. (1990), it was found that MDMA initially reduced milk drinking in mice, but tolerance developed when the drug was administered 5 days a week at a dose of 2.5 mg/kg or 5 mg/kg. In another study by Li et al. (1989), MDMA initially reduced reinforcement rates and increased response rates in rats performing under an interresponse-time-greater-than-72 s (IRT > 72″) schedule. Rats were then administered MDMA at a dose of 6 mg/kg every 12 h for a period of 4 days, and this regimen resulted in sensitization to the acute effects of MDMA, i.e., effects of MDMA

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were greater after the chronic regimen. In a third study done by LeSage et al. (1993) in pigeons, it was demonstrated that MDMA, administered acutely, produced dose-dependent decreases in both accuracy and response rates under a delayed matching to sample procedure. When MDMA was administered chronically, however, tolerance developed to the effects on both accuracy and response rate.

Because MDMA is used intermittently in humans, rather than on a daily basis, one purpose of this study was to determine whether once weekly administration of MDMA would result in tolerance or sensitization to its effects on schedule controlled behavior in rats.

In some cases, both tolerance and sensitization to the effects of a drug can be seen. For example, Rebec and Segal (1980) reported that chronic administration of D-amphetamine enhanced certain aspects of stereotypy, whereas tolerance developed to licking and biting behaviors associated with D-amphetamine administration. Because of these findings, a second part of this study was to determine whether any tolerance or sensitization would occur to the effects of MDMA on a purely spontaneous parameter, i.e., locomotor activity, so that these results could be compared to MDMA's effects on a learned activity such as operant behavior.

2. Materials and methods

2.1. Animals

Sixteen adult male Sprague–Dawley rats weighing 300–320 g served as subjects. They were maintained at 80% of their free-feeding weight. All rats were individually housed in Nalgene cages $25 \times 40 \times 18$ cm in a temperature and humidity controlled room on a 12 h light–dark cycle. Water was available ad libitum.

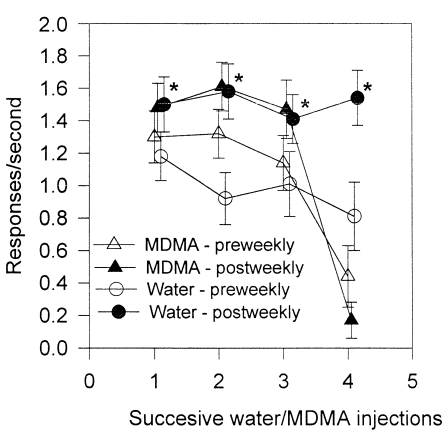

Fig. 1. Response rates after administration of water or MDMA for all rats, before and after weekly treatment. Preweekly refers to the initial cumulative dose–effect curve for MDMA. Postweekly refers to the second cumulative dose-effect curve for MDMA, i.e., after 4 weeks of once weekly dosing with 3.2 mg/kg MDMA. Each point represents the mean for 16 rats. Vertical bars represent standard error of the mean. * indicates significant difference before and after weekly treatment, $P < 0.05$.

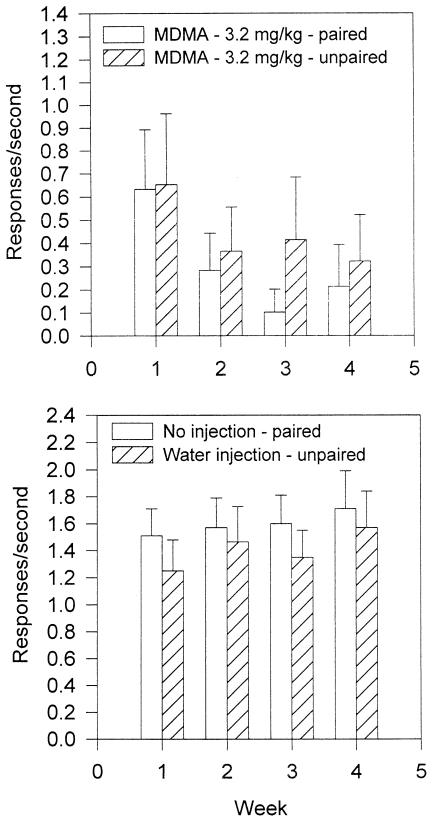

Fig. 2. The top panel of this figure represents rates of responding in both paired and unpaired rats during weekly treatment with MDMA (3.2 mg/kg). The bottom panel of the figure represents rates of responding in both paired and unpaired rats during weekly treatment with water (unpaired) or no injection (paired). The paired group ($n = 8$) received only one injection per week and the injection was always 3.2 mg/kg MDMA. The unpaired group ($n = 8$) also received 3.2 mg/kg MDMA once a week, but they also received a water injection once a week, separated from the MDMA injection by 2 days. On days when the unpaired group got a water injection, rats in the paired group received no injection but were run as a 'test session' (see Section 2.5 for definition of 'test session'). Vertical bars represent standard error of the mean. There are no significant differences in responding over the 4 weeks in either group.

2.2. Apparatus

2.2.1. Operant chambers

The experimental sessions were conducted in standard operant chambers $22 \times 22 \times 28$ cm (Med-Associates, St. Albans, VT, USA). Each chamber was equipped with three response levers located 8 cm above the floor and separated 1 cm from each other. Three 7-W white lights were mounted on the intelligence panel 5 cm above each response lever. A receptacle for food delivery was located on the opposite wall. A reinforcer in the form of a 45 mg food pellet (Bio-Serv) was delivered when the subject completed the schedule requirements. The operant chambers were enclosed in sound attenuating outer chambers with

white masking noise continually present, and a fan for ventilation. The schedule requirements and collection of data were controlled through a microcomputer (Med-Associates) interface and software. Sessions were conducted 5 days a week.

2.2.2. Locomotor activity chambers

Each of the four Med-Associates locomotor activity chambers (Med-Associates) consisted of $43 \times 43 \times 30$ cm Plexiglas. An array of photo beams was situated 2 cm above the Plexiglas floor on each wall. When sampled through the computer, these beams were used to define the $X-Y$ position of the animal with 4 cm resolution and also to measure distance traveled in centimeter. Rearing (vertical counts) was also detected by two photocell strips located 8 cm above the floor of the chamber on opposing walls. Every 100 ms, the computer recorded status of the activity in the chamber through the sensitivity of the photo beams. If any change occurred from the previous reading of the photo beams, the current status of all the beams was stored together with the number of 100 ms intervals since the previous reading.

2.3. Drugs

MDMA HCl was kindly supplied by Dr. Jack DeRuiter, Department of Medicinal Chemistry, Auburn University (Auburn, AL, USA). The drug was dissolved in distilled water and all solutions were made on the day of the experiment. The drug was administered i.p. in a volume of 1 ml/kg. All doses are expressed as the salt.

2.4. Procedure

The subjects were handled and weighed for a period of 2 days before the beginning of training. Subjects were initially exposed to a fixed time 1 min procedure that resulted in delivery of a food pellet at the end of each minute. A response on any lever also resulted in delivery of a food pellet. The fixed time was doubled each day until rats learned to press the lever for reinforcement. As soon as rats learned to press the lever for reinforcement they were put on a fixed ratio 1 (FR 1) schedule of reinforcement. The schedule ratio was gradually increased for all the subjects until FR 20 and they were maintained at this schedule throughout the experimental period.

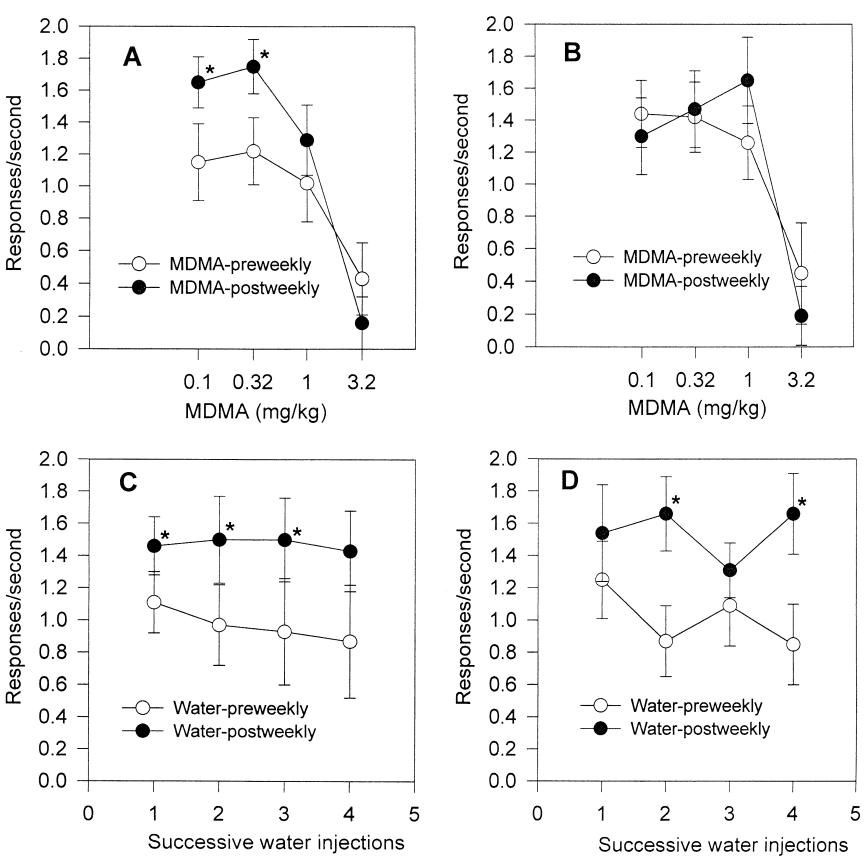

Fig. 3. The top panels represents the effects of MDMA for rats in the paired group (panel A) and the unpaired group (panel B) before and after weekly treatment with 3.2 mg/kg MDMA. The bottom panel shows the response rates with four water injection for rats in the paired group (panel C) and the unpaired group (panel D) before and after weekly treatment. Vertical bars represent standard error of the mean. * indicates a significant difference ($P < 0.05$) in response rate when preweekly rates are compared to postweekly rates. For definition of paired and unpaired groups, refer to the legend of Fig. 2. For definition of preweekly and postweekly, refer to the legend of Fig. 1.

2.5. Experimental protocol

Prior to the administration of water or drug, response rates were stabilized such that there was no more than 5% variation from day to day over a period of 2 weeks. Rats then received a 'test' dosing regimen that consisted of four consecutive water injections. After each water injection there was time out period of 10 min, during which the rats were returned to their home cages. At the end of each time out, the rats were then put in the operant chamber for a test session of 5 min or 5 reinforcers, whichever occurred first. This series of water injections was repeated twice before drug testing and each water 'test' series was separated by 1 week.

One week following the second water 'test', cumulative doses of MDMA (0.1 to 3.2 mg/kg) were administered to all rats. The first injection of MDMA was 0.10 mg/kg followed by a second injection of 0.22 mg/kg (cumulative 0.32 mg/kg), a third injection of 0.68 mg/kg (cumulative 1.0 mg/kg), a fourth and final injection of 2.2 mg/kg (cumulative 3.2 mg/kg). The schedule of injection, time out and run time was identical to those during the water 'test' series, as described above, i.e., rats were injected and tested after each cumulative dose of MDMA. One week after the dose response curve for MDMA was generated, the rats were separated into two groups, paired and unpaired, 8 rats each. The groups were chosen to include rats that were similarly sensitive to the effects of MDMA. All rats were then injected once weekly with MDMA at a dose of 3.2 mg/kg. On these weekly injection days, rats were injected, returned to the home cage for 10 min and then placed in the operant chamber for a test session of 5 min or 5 reinforcers, whichever occurred first. Both groups received weekly MDMA every Friday for a period of 4 weeks. Rats in the unpaired group also received a single water injection each week for 4 weeks. Water injections were administered on Wednesdays and the session that followed 10 min later was also a test session. Rats in the paired group were not injected on Wednesday but were placed in the operant chambers for a test session. The reason for the additional water injection in the unpaired group was to rule out any possible conditioning effects of the MDMA injection. One week following the fourth weekly injection of MDMA, the cumulative dose effect

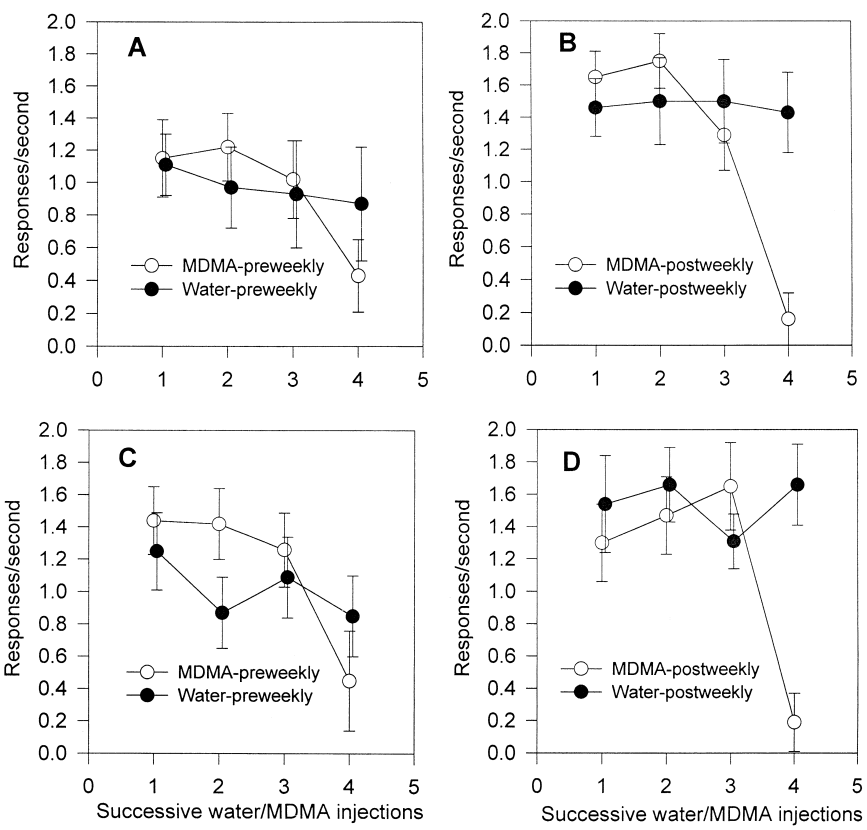

Fig. 4. The top panels (A and B) represents the effects of injections of both MDMA and water for rats in the paired group before and after weekly treatment with MDMA. The bottom panels (C and D) shows effects of injections of both MDMA and water for rats in the unpaired group before and after weekly treatment with MDMA. Successive MDMA injections refer to cumulative doses of 0.1, 0.32, 1.0 and 3.2 mg/kg, respectively. Vertical bars represent standard error of the mean. For definition of paired and unpaired groups, refer to the legend of Fig. 2. For definition of preweekly and postweekly, refer to the legend of Fig. 1.

curve for MDMA was regenerated and the following week, four consecutive water injections were once again administered.

During the chronic treatment period, four subjects in unpaired group were also assessed for locomotor activity. The rats were placed in the locomotor activity chamber immediately after water or MDMA tests for a period of 60 min. During this time, distance traveled and vertical counts were measured, and statistics were gathered for each 15′ component for weeks 1 and 4.

2.6. Data analysis

All data were analyzed using analysis of variance and *t*-tests and significance was set at $P < 0.05$.

3. Results

3.1. Dose–effect curve for MDMA and water

Fig. 1 shows the effects of MDMA and water on operant responding both before and after once weekly treatment for all the rats combined. As can be seen from the figure, rates of responding after water injections were significantly higher after once weekly treatment with MDMA. MDMA tended to increase rates in these grouped data at the lower doses and clearly decreased rates at the highest dose. Rates for MDMA in the grouped data were not significantly different before and after weekly treatment.

3.2. Weekly administration of MDMA

The top panel of Fig. 2 shows the response rates of the rats in both the paired and unpaired groups during the weekly administration of 3.2 mg/kg MDMA. It can be seen that there was no tolerance to the rate decreasing effects of MDMA. In fact, all the rats appeared to become more sensitive to MDMA. The bottom panel of Fig. 2 shows the response rates in both the groups, with no injection in the paired group and a water injection in the unpaired group, during the weekly treatment. There was a slight increase in rates over the 4 weeks in both groups.

3.3. Dose–effect curve for MDMA

The top panels of Fig. 3A and B show the dose–effect curves for MDMA for both groups before and after weekly treatment. At lower doses there was a significant increase in response rate after weekly treatment in paired group rats (Fig. 3A, closed circles), whereas there was little to no

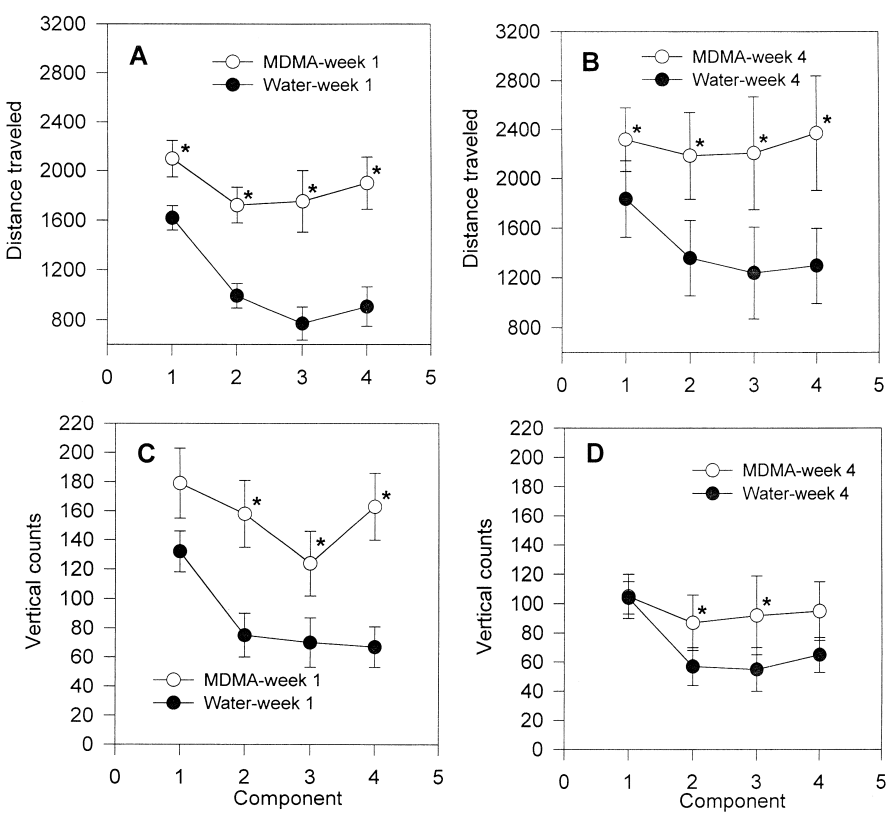

Fig. 5. The top panels compare distance traveled (in cm) after MDMA and water on week 1 (panel A) and week 4 (panel B) of the weekly treatment. The bottom panels represent vertical counts (rearing) on week 1 (C) and week 4 (D). Each component represents 15 min of the 60 min session. Vertical bars represent standard error of the mean. * indicates significant difference ($P < 0.05$).

increase in the response rate of the rats in the unpaired group at the same doses (Fig. 3B, closed circles). The postweekly response rate at the highest dose was not significantly different from the preweekly response rate in either group.

3.4. Response rates after successive water injections

The bottom panels of Fig. 3C and D show the response rates after successive water injections, before and after weekly treatment. Water injections produced significant increases in rates of responding in both grups after weekly treatment with 3.2 mg/kg MDMA (Fig. 3C and D, closed circles) as compared to preweekly water injections (Fig. 3C and D, closed circles).

3.5. Comparison of the response rates between MDMA and water

The comparison of the response rates after cumulative injections of MDMA and four successive water injections, is shown in Fig. 4. Fig. 4A and B illustrate the comparison of the rates in the paired group, with Fig. 4A representing MDMA and water before weekly injection of 3.2 mg/kg MDMA, and Fig. 4B representing MDMA and water after weekly injection of 3.2 mg/kg MDMA. Fig. 4C and D represent the same comparisons in the unpaired group. In both groups low doses of MDMA slightly increased responding as compared to water injections before the weekly regimen. After once weekly treatment with 3.2 mg/kg MDMA, the response rate after cumulative injections of MDMA and successive water injections was higher in both groups than it was before the weekly dosing regimen was initiated. The highest dose of MDMA eliminated responding in the majority of rats both before and after weekly administration of 3.2 mg/kg MDMA, but this effect was more pronounced after weekly treatment in both groups.

3.6. Locomotor activity

Fig. 5 shows the effects of 3.2 mg/kg MDMA and water on locomotor activity in the unpaired group. MDMA significantly increased distance travelled and vertical counts on both week 1 and week 4 of the weekly dosing regimen when compared to a water injection. Fig. 6 shows that, by the end of week 4, distance traveled increased over time, irrespective of whether it was a water injection or an injection of 3.2 mg/kg MDMA. In contrast, vertical counts decreased over time.

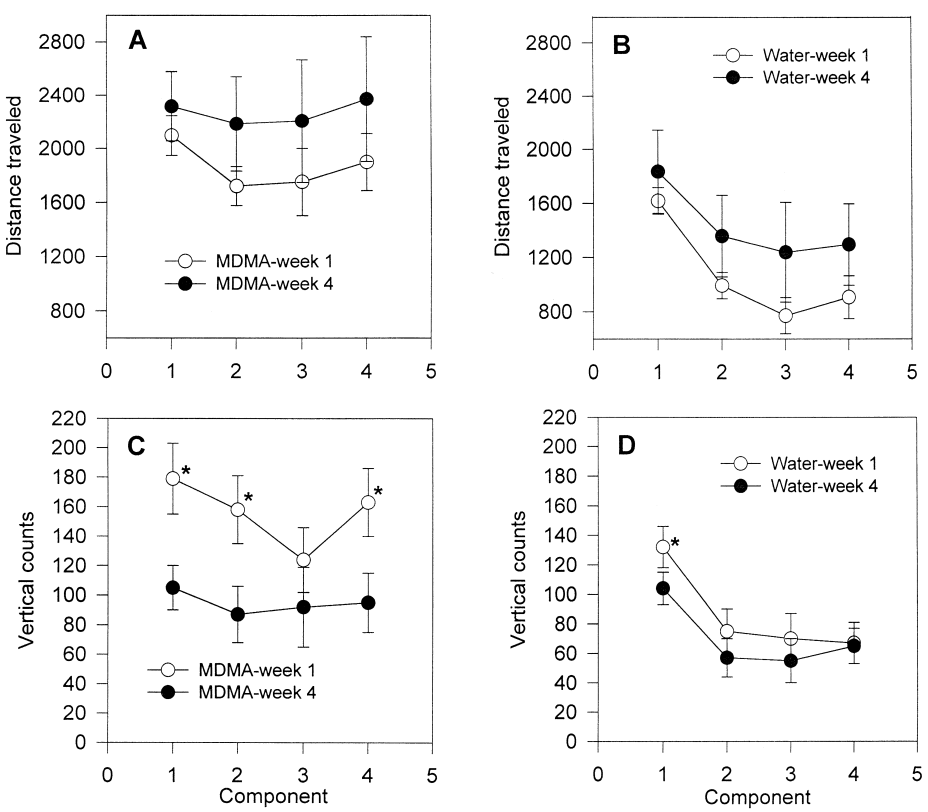

Fig. 6. The top panels show distance traveled (in cm) on week 1 vs. week 4 after the weekly injection of MDMA (3.2 mg/kg) (panel A) or water (panel B) in the unpaired group. Vertical counts (rearing) are shown in the bottom panels for MDMA (C) or water (D). Each component represents 15 min of the 60 min session. Vertical bars represent standard error of the mean. * indicates significant difference ($P < 0.05$).

4. Discussion

In the present study, before rats were separated into two groups, cumulative injections of MDMA increased response rates at low doses and decreased response rates at the highest dose when compared to successive water injections (see Fig. 1). These results are similar to those of Miczek and Haney (1994) who also found decreases in response rates at high doses of MDMA on an FR 30 schedule in mice. In contrast, Miczek and Haney (1994) did not report rate-increasing effects of MDMA at low doses.

As seen in Fig. 1, once weekly treatment with 3.2 mg/kg MDMA shifted the MDMA dose–effect curve upward at low doses and downward at the highest dose. As also shown in Fig. 1, however, rates of responding were also increased after successive water injections following weekly treatment with MDMA, and in fact, rates of responding after the first three water injections are virtually identical to rates of responding after the first three doses of MDMA. Thus it is only at the highest dose of MDMA that rats appeared to be more sensitive to the rate decreasing effects of this drug.

The weekly treatment results (Fig. 2) indicated no development of tolerance during once weekly administration of 3.2 mg/kg MDMA. The lack of tolerance seen in the weekly sessions may well have been because the session time was too short (5 min). Indeed, when rats were placed in the locomotor activity chamber immediately after the weekly operant test sessions, there was an increase in distance traveled from week 1 to week 4, suggesting either tolerance or non-specific hyperkinesis.

When the paired group and unpaired group are analyzed separately, however (Figs. 3 and 4), it is evident that there was no change in rates of responding after low doses of MDMA in the unpaired group, compared to a significant increase at the same doses in the paired group after the weekly regimen. Because the paired group received only weekly MDMA injections, whereas the unpaired group received both water and MDMA injections each week, it is possible that the increased responding in the paired group is due to a conditioning effect. This increase in response rates after weekly treatment with MDMA is suggestive of a compensatory conditioned response (Goudie and Demellweek, 1986). For example, the presentation of MDMA (unconditioned stimulus) is always paired with injection (conditioned stimulus) and the conditioned stimulus subsequently elicits the compensatory conditioned response, i.e., increase in rates of responding. Thus in the paired group in which injection and MDMA were always paired, low doses of MDMA led to a compensatory response in terms of increased responding. It was observed by Rescorla (1968) that acquisition of a conditioned response is retarded if the conditioned stimulus is not always paired with the unconditioned stimulus. Since the rats in the unpaired group only received MDMA 50% of the time during weekly treatment, any increase in response rates, was retarded when compared to the paired group.

Another interesting finding in the current study was the significant increase in rates of responding in both groups when successive water injections were administered following weekly treatment with MDMA. This increase in response rates was not expected and might suggest that the baselines were not stable before the weekly regimen. This is unlikely, however, because the animals had maintained a stable baseline performance for 2–4 weeks before testing. One possible explanation is that MDMA itself may have increased overall activity, possibly through an unknown neurotoxic effect. At least one other study has reported similar effects. For example, Dafters (1995) showed that chronic treatment with MDMA resulted in an augmentation of hyperthermia and hyperkinesis in rats.

In terms of locomotor activity, our rats had an increase in distance over the 4 week regimen, at the same time that vertical counts decreased. These effects were seen after both water and MDMA injections, suggesting a nonspecific increase in activity. These results are similar to those of Spanos and Yamamoto (1989) who found that intermittent administration of MDMA resulted in augmented hyperlocomotion. These investigators found that the time course of hyperlocomotion seen in their study was paralleled by the time course of MDMA-induced dopamine release. Though the results of our present study agree with those of Spanos and Yamamoto (1989), it is hard to say whether the hyperlocomotion seen was due to the release of dopamine since concurrent experiments to determine the dopamine content in the brain of the rats were not performed.

In summary, it would appear that even once weekly treatment with a fairly low dose of MDMA produces changes in both operant and locomotor behavior. These changes may be due to a pharmacological and/or a behavioral or associative mechanism, although the precise nature of the behavioral mechanism cannot be characterized.

Acknowledgements

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