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Effects of Methadone on Behavior Maintained by Fixed Ratio Reinforcement Schedules[1]

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MIDDAUGH, L. D. AND C. A. SANTOS, III. *Effects of methadone on behavior maintained by fixed ratio reinforcement schedules.* PHARMAC. BIOCHEM. BEHAV. 8(5) 521–526, 1978. – The effects of subcutaneous injections of methadone hydrochloride (0.75 mg, 1.5 mg, and 2.5 mg/kg) on lever pressing maintained by fixed ratio schedules of reinforcement were examined in C57BL/6J and DBA/2J mice. Response output over a 30 min session decreased as a function of increasing drug dose when reinforcement was delivered for every 5 responses. Increasing the response to reinforcement ratio from 5 to 20 in a second experiment doubled and nearly quadrupled responding by DBA and C57 mice, respectively. Injecting animals maintained on this schedule with methadone reduced responding to the same extent as that observed in the first experiment when response rates were lower. Hence, these experiments provide no support for the effect of methadone being rate-dependent. In both experiments methadone disrupted responding of DBA more than that of C57 mice. This finding is consistent with a previous report that other narcotic analgesics were more potent analgesics for DBA than for C57 mice. From these studies it appears that the opposite changes in locomotor activity upon an exposure to narcotic analgesics does not generalize to behavior under control of reinforcing stimuli; and that both the effects obtained in the present study and the analgesic action are dissociated from the effects of these drugs on locomotor activity.

Operant behavior Methadone Inbred mice

CURRENTLY there are few reports in the literature regarding the effects of methadone on behavior of laboratory animals. Hill *et al.* [8] early reported that methadone injected into rats produced a dose related interruption of conditioned suppression. Thus, rats injected with methadone made more responses in the presence of a signal which had previously been paired with shock than did control animals. In addition, the drug at higher concentrations (3.0 mg–4.5 mg/kg) caused an overall reduction in responding by rats when food was delivered on a variable interval (Mean Interval: 2 min) schedule of reinforcement. Others have reported that methadone disrupts key pecking by pigeons during both components of a combined fixed ratio, fixed interval schedule of reinforcement [7,12]. In the latter two studies low doses of methadone increased response rate under the fixed interval component of the schedule in some animals. In all three studies the effects of methadone were similar to those obtained with morphine sulfate, although it was suggested in one study that methadone was more potent [8].

Most other reported studies regarding the effects of methadone on behavior have utilized some form of activity measure as the dependent variable. Methadone has been reported either to elevate or lower activity levels depending upon the drug dose, the time after injection, and the species or strain of animal tested. Injected into rats, methadone has most frequently been reported to produce a cataleptic response [1, 11, 17]. However, there is one report that low dose of the drug elevate activity and higher doses first lower then elevate activity [5]. The reported biphasic action of methadone on activity of rats is consistent with results obtained following injections of morphine [2,3]. Although it is commonly accepted that injections of methadone or morphine into mice elevate activity [3, 10, 11], it has recently become evident that both of these drugs can either elevate or lower activity levels depending on the particular strain of mouse tested [4, 13, 14].

Recently there has been increasing effort to account for the differences between species [11] or strains [15] in the direction of activity change following injections of narcotic analgesics. The purpose of the experiments reported here was to examine the generality of strain difference in reaction to narcotic analgesics by determining the effects of methadone on behavior under the control of reinforcing stimuli in strains of mice previously reported to have opposite changes in activity following injections of narcotic analgesics. Although narcotic analgesics elevate or lower activity of C57BL/6 (C57) or DBA/2 (DBA) mice, respectively, [4,13], the results of the current study demonstrate that methadone disrupts behavior maintained by fixed ratio schedules of reinforcement for mice of both strains.

EXPERIMENT 1

Experiment 1 was conducted to determine the effects of

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various doses of methadone on lever responding by C57 and DBA mice when reinforcement was delivered for every fifth response (FR5).

Method

Animals. Ninety-day-old C57 and DBA mice, 12 each, were used for this experiment. Mice were obtained from the Jackson Laboratories at 49 days of age. Upon arrival they were housed 4 per cage and maintained in a temperature regulated room (23° ± 2°C) on a 12 hr light:dark cycle. Food and water were available ad lib until the deprivation phase of the experiment at which time the animals were individually housed and fed on a schedule to reduce body weight at 80% ± 5% of ad lib weights.

Apparatus. Animals were tested in six operant chambers enclosed in sound attenuated boxes. The chambers (16 × 16 × 11.4 cm) were constructed by Plexiglas with stainless steel grid floors. A food tray with a 1.9 × 2.5 cm opening was centrally located on one 16 cm wall at floor level. A Lehigh Valley Electronic Model No. 121–03 rodent lever located 4.0 cm to one side of the food tray and 3.0 cm above the floor served as the response indicator. Eight grams dead weight on the lever closed the microswitch. Subsequent release of the switch defined a response and served as input to solid state programming equipment. Responses were cumulated and printed out at 1 min intervals. Food pellets (Noyes, 20 mg) were delivered to the food tray from a dispenser located adjacent to the operant chamber.

Procedure. After seven days on restricted feeding (0.05 g–0.08 g food/g ad lib body weight) which reduced body weight to 80% ± 5% of ad lib feeding levels, each animal was placed in an operant chamber for 15 min on two successive days. During this time, 5 food pellets were initially present in the food tray but the reinforcement mechanism was disconnected. On the following two days, the mice were allowed 15 min per day in the operant chamber during which time each response produced a food pellet. Animals that failed to acquire the response (∿15%) in the two 15 min sessions were allowed additional time in the chamber. After response acquisition (>10 responses in a 15 min period), the mice were run 30 min per day for three days with reinforcement available for each response. At this time the FR5 schedule of reinforcement was initiated. For the remaining time of the experiment animals were run 30 min sessions per day, five sessions per week. Beginning on the sixth session of FR5, animals were injected SC with physiological saline (0.008 ml/g body weight) 5 min prior to each daily session. This procedure was continued throughout the experiment except on the third day of each 5 session block which was used for drug assessment.

Drug tests began after 7 days of saline injection and occurred at weekly intervals over a four week period. On drug test days mice were injected with either saline or one of three doses of methadone hydrochloride (0.75 mg,

TABLE 1

LEVER RESPONSES BY C57BL/6 AND DBA/2 MICE MAINTAINED ON FR5 SCHEDULE OF REINFORCEMENT FOLLOWING INJECTIONS OF SALINE OR METHADONE HYDROCHLORIDE*

Strain	Drug Test Dose (mg/kg)	Response Output: Pre-Drug Test Day $\bar{X}$ ± SEM	Drug Test Day† $\bar{X}$ ± SEM	Post-Drug Test Day $\bar{X}$ ± SEM
C57B1/6	0.00	284 ± 15	298 ± 18‡	287 ± 21
	0.75	285 ± 13	265 ± 18	294 ± 15
	1.50	289 ± 16	208 ± 20	289 ± 16
	2.50	289 ± 12	92 ± 22	267 ± 16
DBA/2	0.00	227 ± 16	237 ± 12§	205 ± 21
	0.75	221 ± 12	172 ± 12	212 ± 12
	1.50	225 ± 18	134 ± 18	211 ± 21
	2.50	226 ± 17	34 ± 12	216 ± 16

*Male C57BL/6 and DBA/2 mice, 12 each were injected SC with saline 5 min prior to 30 min lever-press sessions on Pre- and Post-Drug Test Days. On Drug Test Days they were injected with methadone at doses of 0 (saline), 0.75 mg, 1.5 mg or 2.5 mg/kg. Results are expressed as mean $\bar{X}$ ± SEM number of responses generated during the 30 min sessions.

†A *2(Strain)* × *4(Drug Dose)* analysis of variance established significant *Strain* and *Drug* effects.

‡0, 0.75>1.5>2.5 (Newman-Keuls test for multiple comparison, $p<0.01$)

§0>0.75, 1.5>2.5 (Newman-Keuls test, $p<0.01$)

1.5 mg or 2.5 mg/kg body weight) 5 min prior to the session. Over the four drug test days, each animal received each dose of methadone and one saline injection. Since drug assessment occurred on the third day of each 5 day block, at least 7 days intervened between each drug injection. In addition, the order in which the various doses were given was balanced across drug assessment days according to the following scheme. Three mice from each strain were arbitrarily assigned to one of four groups. The order of methadone doses (mg/kg) was: for Group 1 – 0, 0.75, 1.5, 2.5; for Group 2 – 0.75, 1.5, 2.5, 0; for Group 3 – 1.5, 2.5, 0, 0.75; and for Group 4 – 2.5, 0, 0.75, 1.5.

Statistical Analysis. Data were analyzed using 2 (Strain) × 4 (Drug Dose) analyses of variance with repeated measures on the drug factor. Statistical significance of group differences was assessed using the Newman-Keuls test for multiple comparisons [20].

Results

Mean responses generated during the 30 min sessions on days prior to drug testing, on days of drug testing and on days following drug testing are summarized for both strains in Table 1. Inspection of these means indicates that within each strain the number of responses per session during the days prior to and following drug testing was similar regardless of the particular dose injected on the intervening drug testing day. This result indicates that the effect of methadone observed on drug test days was absent 24 hr after injection. It is also evident that lever responding is reduced as a function of drug dose in both strains. For C57 mice, doses of 0.75 mg, 1.5 mg and 2.5 mg/kg reduced mean response output during 30 min sessions by 11%, 30% and 69% compared to mean responses following saline injections. Thse doses injected into DBA mice reduced responding by 27%, 43% and 86%. Finally, it is evident that C57 mice emit more responses over the 30 min session than do DBA mice. The noted Drug and Strain effect was statistically supported by an analysis of variance (Strain, $F(1,22) = 14.88$, $p \leq 0.1$; Drug Dose, $F(3,66) = 72.39$, $p \leq 0.01$). This analysis, however, did not support a Strain × Drug interaction, $F(3,66) = p > 0.1$. Since the Strain × Drug interaction mean square is influenced by the reduced level of responding by DBA mice without drug exposure as well as during drug exposure, the possibility of a strain difference in reaction to the drug is not adequately assessed by this analysis.

To provide a more comprehensive examination of the influence of methadone on this behavior, within session response distributions under the various drug conditions are plotted in Fig. 1 as mean cumulative responses over 5 min intervals throughout the sessions. Inspection of these graphs indicates that DBA mice were more severely affected by the drug than were C57 mice. This is evident within 5 min of testing. During this 5 min period, mean response output following saline injections was similar for both strains: 68 ± 5 and 67 ± 5 for C57 and DBA mice, respectively. The similar basal response rate for both strains when injected with vehicle allows assessing possible strain differences in reaction to the drug. Analysis of the variance of the data during this time interval again established significant Strain and Drug effects. In addition, a marginally significant Strain × Drug interaction, $F(3,66) = 2.31$, $p < 0.1$, was obtained. Compared to mean response output over the 5 min period following injections of saline, injections of 0.75 mg, 1.5 mg and 2.5 mg/kg methadone reduced responding 1%, 24% and 59% for C57 mice and 18%, 34% and 91% for DBA mice. Newman-Keuls tests for significance of mean differences under the various drug conditions within each strain established that only the high dose significantly reduced responding by C57 mice. All drug doses, however, significantly lowered responding by DBA mice.

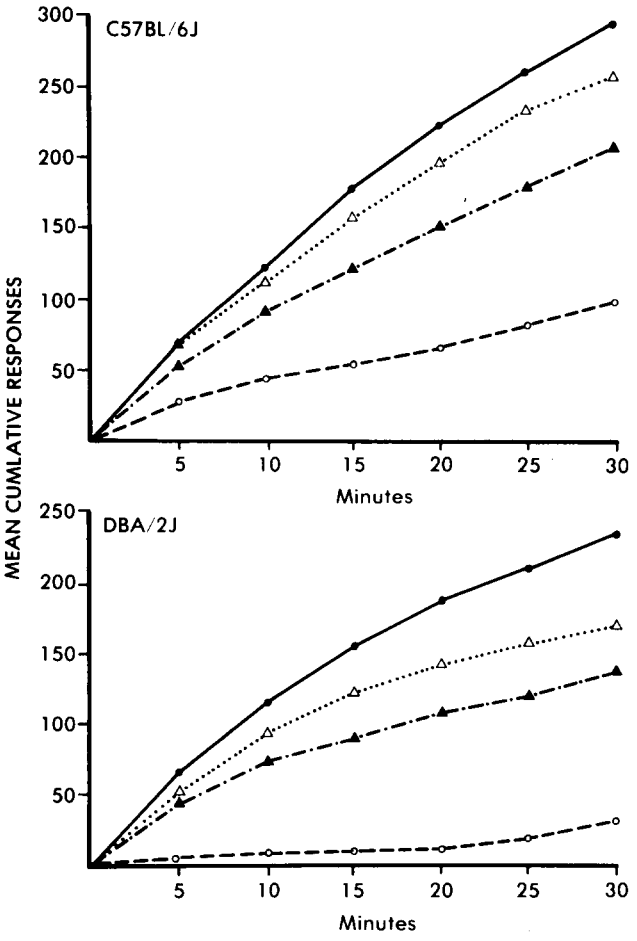

FIG. 1. Mean cumulative lever responses generated over a 30 min testing period following SC injections of saline (•), or methadone at 0.75 mg (△), 1.5 mg (▲) or 2.5 mg (○)/kg into C57BL/6J (upper graph) or DBA/2J (lower graph) mice. Animals were injected 5 min prior to testing and the lever response was maintained by food reinforcement for every five responses.

EXPERIMENT 2

The results of the first experiment demonstrated that injections of methadone disrupted lever responding by mice maintained on the FR5 schedule of reinforcement. There is evidence originally published by Dews [6] and recently reviewed by Sanger and Blackman [16] that the effect of drugs on response rate is often influenced by the response rate under drug free conditions. The purpose of the second experiment was to determine if the disruptive effect of methadone observed in the first experiment would be altered if drug free response levels were higher than those obtained under the FR5 schedule of reinforcement.

Method

Animals and apparatus. The apparatus was that used and described for Experiment 1. C57 and DBA mice, 5 each, were housed as described for Experiment 1.

Procedure. Food deprivation and response acquisition were as described for Experiment 1, however, in the present experiment animals received daily 30 min sessions 7 days per week rather than 5 days per week as in Experiment 1. After 5 days of responding under the FR5 schedule, the ratio was increased to 20 responses per reinforcement (FR20). The mice were injected with saline 5 min prior to each daily session beginning on the sixth day of FR20 schedule and the procedure was continued until daily response output had stabilized. In this experiment, we assessed the effects of only the intermediate dose of methadone (1.5 mg/kg) and used a within subjects design with each animal serving as its own control. Each animal received two SC injections of the drug separated by three days on which saline was injected prior to the session. Drug effects were assessed by comparing mean response output following drug injections with mean response output during the three days prior to drug injections.

Results

Mean responses generated during 30 min sessions after saline or methadone injections are summarized in Fig. 2. Baseline means (B_1 and B_2) reflect responses averaged over the three daily sessions prior to drug testing. Response output remained constant within each strain across the experiment as evidenced by similar mean responses during days of saline injections (B_1 and B_2). Mean response output on days of methadone injections (D_1 and D_2) is reduced compared to baseline response means for both strains on both of the drug test days. Comparisons of means during test days with preceeding baseline days via t tests for correlated means established that these reductions were statistically significant, C57: B_1 vs D_1, $t(4) = 12.50$, $p \leq 0.01$; B_2 vs D_2, $t(4) = 7.78$, $p \leq 0.01$; DBA: B_1 vs D_1, $t(4) = 6.248$, $p \leq 0.01$; B_2 vs D_2, $t(4) = 5.451$, $p \leq 0.01$.

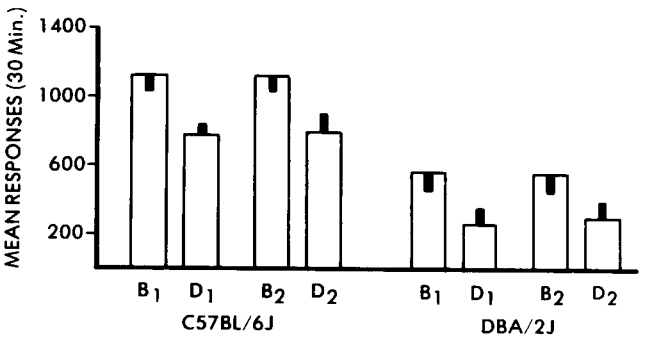

FIG. 2. Lever responses made by C57BL/6 and DBA/2J mice over a 30 min testing period following SC injections of saline (B_1 and B_2) or 1.5 mg/kg methadone (D_1 and D_2) when lever responding was maintained by food reinforcement for every 20 responses. The bars represent means (5 per group) and the vertical lines, standard error of the mean. Animals were injected 5 min prior to testing.

To determine the influence of strain and response rate on the disruptive effects of methadone, data from this experiment were compared with those from animals of the first experiment injected with saline or methadone (1.5 mg/kg) when responding was maintained by the FR5 schedule. Data collected under these conditions are summarized in Table 2. As noted, increasing the response to reinforcement ratio from 5 to 20 doubled and nearly quadrupled response output by DBA and C57 mice respectively. In spite of the higher response rate under the FR20 sechedule, the disruptive effect of methadone was approximately the same as when response rates were lower (i.e., under the FR5 schedule). To provide a statistical

TABLE 2

LEVER RESPONSES BY DBA/2 and C57BL/6 MICE MAINTAINED ON FR5 OR FR20 SCHEDULES OF REINFORCEMENT FOLLOWING INJECTIONS OF SALINE OR METHADONE HYDROCHLORIDE*

Strain	Reinforcement Schedule	Saline $\bar{X} \pm$ SEM	Methadone (1.5 mg/kg) $\bar{X} \pm$ SEM	Percent Reduction
C57BL/6	FR5	298 ± 18	208 ± 20	30
	FR20	1111 ± 79	796 ± 90	28
DBA/2	FR5	237 ± 12	134 ± 18	43
	FR20	559 ± 106	289 ± 96	48

*This table summarizes data generated by animals in Experiment 2 maintained on an FR20 schedule of reinforcement and by animals in Experiment 1 maintained on an FR5 schedule of reinforcement during 30 min sessions following injections of either saline or methadone (1.5 mg/kg). The data indicate that lever responding is disrupted to about the same degree regardless of the base response rates generated under the saline condition and that DBA/2 mice are more severely disrupted by methadone than are C57BL/6 mice. (See text for statistical support.)

assessment of the influence of schedule or strain on the disruptive effects of methadone, response output following injections of methadone was divided by response output following injections of saline for each animal and the ratios were analyzed in a 2 (Strain) x 2 (Schedule) analysis of variance. The results of the analysis established only a significant Strain effect, $F(1,30) = 4.375$, $p<0.05$, with methadone being more disruptive on responding by DBA mice. The Schedule effect and the Schedule x Strain interaction were not significant.

DISCUSSION

The results of this study demonstrate: (1) that methadone hydrochloride injected into DBA or C57 mice disrupts behavior maintained by fixed ratio schedules of reinforcement; (2) that the disruptive effect of the drug appears to be unrelated to basal response rates; and (3) that behavior of DBA mice is more disrupted by the drug than that of C57 mice.

The disruptive effect of methadone on behavior under the control of reinforcing stimuli delivered on fixed ratio schedules observed in this study is consistent with previous reports on the effect of this drug on behavior of pigeons maintained on fixed ratio schedules [7,12]. Although there are no reports on the effects of methadone on behavior of rodents maintained on fixed ratio schedules, disruptive effectives have been reported when behavior was maintained by a variable interval schedule [8]. Low doses of morphine (1 mg/kg), another narcotic analgesic, injected into rats, however, has been reported to elevate responding maintained by fixed ratio schedules [19]. In the same study, higher doses (3 mg and 6 mg/kg) were found to lower responding. The morphine induced elevated responding under fixed ratio schedules, however, is not consistent with results obtained using pigeons and the results were obtained on only three animals. In the current study we did not observe elevated responding with any of the three doses of methadone tested. Both of the higher doses (1.5 mg and 2.5 mg/kg) produced significant disruption of response output over the 30 min sessions for both strains of mice. In addition, the low dose (0.75 mg/kg) disrupted response output of DBA mice.

As expected, increasing the ratio requirement from FR5 to FR20 elevated responding. However, the elevation was not as extensive for DBA (approximately two-fold) as for C57 (approximately four-fold) mice. In spite of the higher basal response rates under the FR20 schedule, methadone disrupted responding to about the same degree as when behavior was maintained by the FR5 schedule (64% and 65% of control levels for FR5 and FR20, respectively). Thus, within the response rate limits of the current study, there is no evidence to suggest that the disruptive effect of methadone is rate dependent. This finding is in accord with the recent review by Sanger and Blackman [16] who found very little empirical support for the rate dependency hypothesis regarding the behavioral effects of narcotic analgesics. Although DBA mice in the current study maintained on the FR20 schedule showed a slightly greater reduction following injections of methadone than those maintained on the FR5 schedule, this could be due to reduced reinforcement on the higher schedule since these animals received approximately half the number of reinforcements.

The strain differences in reaction to methadone observed in this study were not striking when compared to those reported when locomotor activity is the dependent variable. We previously reported that injections of methadone elevated and lowered locomotor activity of C57 and DBA mice, respectively [13]. This finding has also been observed following injections of morphine [4]. In the current study, however, methadone disrupted behavior under control of reinforcing stimuli in both strains of mice. Hence, the opposite effects of methadone on locomotor activity of the two strains does not generalize to the lever response maintained by fixed ratio schedules of reinforcement. It occurred to us that that drug effects on lever responding could be secondary to drug induced changes in activity since either heightened locomotor activity observed in C57 mice or reduced locomotor activity observed in DBA mice following injections of higher doses of methadone are both incompatible with the lever response. Two factors, however, negate this interpretation. First, we have not been able to observe activity changes in either strain of mouse until a dosage of 7.0 mg/kg at which C57 mice have elevated activity and DBA mice have lowered activity. Second, if the lever response decrement following injections of methadone were secondary to drug induced changes in locomotor activity, response output under drug should be approximately the same under both schedules of reinforcement. This, however, was not the case. Response output by drugged animals maintained on the FR20 schedule was higher than that of animals maintained on the FR5 schedule both under drug and drug-free conditions. Thus, it appears that the effect of methadone on behavior maintained by reinforcing stimuli is dissociated from its effects on locomotor activity.

The strain difference observed was the degree and perhaps the time course of disruption. Methadone reduced responding to a greater extent in DBA than C57 mice and the reduction occurred earlier in the session. By 10 min after injection (5 min of responding), DBA mice injected with all three doses had lowered response output whereas only the highest dose reduced responding by C57 mice. By the end of the 30 min session, however, the two higher doses reduced response output in C57 mice. Certainly the most convincing evidence for a strain difference in reaction to methadone is the results obtained during the first 5 min of responding under the FR5 schedule. During this time, response rates were similar for animals of both strains following saline injections, however, DBA mice injected with methadone responded at much lower rates than C57 mice at each of the three doses. The strain difference in reaction to methadone was also apparent when strain differences in basal response rates were artificially balanced by calculating ratios of responses under drug conditions to responses made following saline injections. An analysis of the variance of these ratios indicated a highly significant strain effect in reaction to methadone at 1.5 mg/kg.

The more pronounced effect of methadone on DBA than on C57 mice in the current study is compatible with previous reports that other narcotic analgesics (e.g., morphine and heroine) have a greater analgesic effect on DBA than C57 mice [4]. It has also been reported that the analgesic effect but not the locomotor effect of morphine is dependent upon an intact limbic system since septal lesions attenuate the analgesic response but do not alter morphine induced running [14]. Other evidence has established that septal lesions increase responding on fixed ratio schedules of reinforcement [9]. Hence, it is possible that similar

neural mechanisms might mediate the response decrement observed in the current experiments and the analgesic effects of narcotic analgesics; and that both effects are mediated by neural mechanisms different from those mediating the effect on locomotor activity.

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