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Chronic methamphetamine increases fighting in mice

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Abstract

A propensity for violent behaviors to develop in chronic methamphetamine (METH) abusers has been noted. The idea that increased aggressiveness might result from chronic METH administration was tested in mice after chronic (long-term intermittent, 8 weeks) or single exposures to the drug. A single injection of METH (6 mg/kg) did not augment fighting. In contrast, chronic METH administration significantly increased the number of animals that initiated bite attacks. This regimen also shortened the latency before the first attack. Latency before the first attack was shorter at 20 h after the METH injection than at 15 min after injection. Locomotor activity was not different at 20 h after METH injection, indicating that increased fighting was not secondary to METH-induced hyperactivity. METH-induced increases in fighting were not related to the duration of persistent sniffing after the initial encounter with an intruder since the duration of this behavior was significantly increased at 15 min after METH but not at 20 h post drug. These results indicate that repeated injections of METH can increase fighting behaviors and also alter social interactions in mice. Thus, intermittent administration of METH might be useful as a pharmacological model to study the biochemical and molecular bases of aggressiveness.

Keywords: Substance abuse; Behavior; Aggressiveness; Social interaction; Psychostimulant; Methamphetamine; Amphetamine

1. Introduction

Methamphetamine (METH) abuse is reported to be associated with a high incidence of violence (Carey and Mandel, 1968; Ellinwood, 1971a; Hawks et al., 1969; Szuster, 1990). For example, many METH abusers seen in psychiatric emergency service have been reported to have long histories of aggression toward others (Szuster, 1990). Nevertheless, because these patients often suffer from other personality disorders (Chen et al., 1999), it is not clear if increased aggressiveness might be due to METH-induced changes or might be secondary to a premorbid proneness to aggressive behaviors due to psychopathology.

Because longitudinal analysis of behavioral deviations in human METH abusers might be limited by ethical and practical considerations, the development of animal models could provide valuable tools in the investigations of aggressiveness and its relatedness to chronic METH intake. Although animals are commonly used to study the biochemical, molecular, and functional neuroanatomy of substance abuse, there is a sparse and inconsistent literature regarding the effects of METH on aggressive behaviors in animals (Crowley, 1972; Maeda et al., 1985; Miczek and O'Donnell, 1978; Shintomi, 1975). Most of these are also limited to acute administration of the drug. Specifically, Shintomi (1975) reported fighting in mice injected with METH (5 mg/kg sc) and placed together with a large number of animals in a limited space. Another group reported that METH lowered the thresholds for defensive attack behaviors elicited by electrical stimulation of the ventromedial hypothalamic (VMH) nucleus in cats (Maeda et al., 1985). Crowley (1972) reported a dose-dependent increase in fighting time in rats after acute administration of METH (up to 1 mg/kg). By contrast, Miczek and O'Donnell (1978) reported no effects of single METH doses below 8 mg/kg on aggressiveness in mice. Reports on the effects of long-term administration of METH on aggressive behaviors were not found in our search of the available literature.

Several studies have also examined the effects of acute administrations of related but chemically and pharmacologically distinct psychostimulants. These include investigations of the effects of methylenedioxymethamphetamine

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(MDMA) and of amphetamine (AMPH) on aggressive behaviors in rodents. For example, a single dose of MDMA, given to mice, caused a reduction of aggression that was also accompanied by a decrease in social investigation (Navarro and Maldonado, 1999). However, acute administration of MDMA caused reduced aggressive behaviors but increased social interactions among rats (Morley and McGregor, 2000). No alterations in social behaviors in rats were reported after repeated injections of AMPH in one study (Sams-Dodd, 1995), whereas a suppression of investigatory and aggressive behaviors was reported in another study (Mitchell and Redfern, 1997).

Based on the human literature and limited animal data, we hypothesized that increased aggressiveness and changes in social interactions noted in chronic METH abusers might be secondary to long-term neuroadaptations to repeated exposures to METH rather than to biochemical and/or physiological responses to acute administration of the drug. To test this idea, the effects of long-term intermittent as well as acute exposures to METH on fighting behaviors were investigated in mice. This study also examined whether or not aggressive behaviors correlated with locomotor activity and/or with the duration of persistent sniffing/following of an intruder after the first encounter with the intruder.

2. Methods

The protocols used in this study were approved by the Animal Care and Use Committee of NIDA IRP. All efforts were made to use the minimal possible number of animals to address the questions of the current study.

Male CD-1 mice (9–11 weeks old) were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were housed in groups of four or five in a cage ($27 \times 16 \times 12$ cm) with free access to food and water. They were maintained on a 12:12-h light/dark cycle (lights on at 7:00 a.m.) at 21 ± 2 °C. Group housing was maintained until 2 weeks before testing for fighting activity. Before being given the injections, mice were habituated to their environment for 1 week. In Experiment 1, mice were randomly assigned to the METH/METH (chronic METH), Sal/METH (acute METH), or Sal/Sal (control) groups.

METH/METH mice received intraperitoneal injections of METH in 0.5 ml of saline at 8 a.m. and 2 p.m. according to the following schedule of escalating METH doses: During the first week, the mice received on Wednesday METH: 1 mg/kg (8 a.m.) and 2 mg/kg (2 p.m.) of METH; on Thursday, they received 3 mg/kg (8 a.m.) and 4 mg/kg (2 p.m.); and on Friday, they received 5 mg/kg (8 a.m.) and 6 mg/kg (2 p.m.) of METH. During the second, third, and sixth to eight weeks, they received two injections of METH (6 mg/kg). They received no injections on the fourth and fifth weeks and there were no injections on weekends.

The chronic METH regimen was designed to bear a certain degree of pseudoparallelism with clinical patterns of METH abuse, which can vary significantly but usually include gradual increases of drug intake to result in the consumption of large doses as well as occasional interruptions of drug use after binges (Kramer et al., 1967).

Control (Sal/Sal) mice chronically received 0.5 ml of saline at the same time as the METH/METH mice received METH. Sal/METH mice chronically received saline like the Sal/Sal mice except the last injection was METH. Thus, the Sal/Sal group represents chronic treatment with saline, while Sal/METH represents chronic treatment with saline followed by a single injection of METH (6 mg/kg). Mice were isolated and single housed after the sixth week when they were 15–17 weeks old.

In Experiment 2, mice were randomly assigned to the METH/METH or Sal/Sal groups and injected with METH or saline, respectively, as in Experiment 1. Mice were isolated and single housed after the fifth week when they were 15 weeks old. They were tested starting on Day 5 of Week 7 and the duration of persistent sniffing/following at the initial encounter with an intruder was observed as described below.

In Experiment 3, mice (12–14 weeks old) were isolated and housed individually for 2 weeks while receiving saline injections (two times a day, 0.5 ml). They were randomly assigned to two groups Sal/METH/Sal/Sal and Sal/METH/Sal/METH at 14–16 weeks of age. One week later, mice in both groups received a single dose of 6 mg/kg METH. After another 1 week, both groups received saline. Six weeks later, Sal/METH/Sal/Sal group received saline while Sal/METH/Sal/METH received METH (6 mg/kg).

Behavioral tests were conducted between 8:00 a.m. and 2:00 p.m.

2.1. Assessment of fighting activity

Fighting activity was examined quantitatively using the ‘‘resident–intruder’’ paradigm (Miczek and O’Donnell, 1978). Tests were performed in the ‘‘resident’’ mouse cage. We measured fighting activity using two measures: The first one was the fraction of animals that initiated a bite attack, and the second one was latency before the first attack bite. Mice were single housed for 2 weeks before tests. Intruder mice were housed in groups of four. Intruders were used for testing only once. Intruder mice (total 294) were males and of the same age and from the same shipment as the tested mice. Latency before the bite attack was measured as time between the placement of the intruder in the resident cage and the first bite attack. Note that the latency for mice that did not initiate a bite attack was assumed to be 900 s, which corresponded to the total time of observation.

Pilot studies had determined that transfer of cages with mice to another room significantly increased aggressiveness both of the tested mice and of the intruders. To avoid confounds caused by stress related to moving, tests were performed in the same room where the mice were housed. Preliminary observations had also shown that changing

bedding in cages shortly or a few days before tests almost completely eliminated differences between "resident" and "intruder" behaviors. Therefore, bedding in cages was changed once a week. Tests for aggressiveness were performed on Day 6 or 7 after changing the bedding. Pilot studies had also determined that different shipments of CD mice received from the same source (The Jackson Laboratory), but at different times, exhibited different aggressiveness, both before and after METH. Overall, 17- to 18-week-old mice tested in May through September (born January through May) were more aggressive than those tested in November/December (born July/August). The May/September mice often engaged in fights when housed in groups even without METH injections. The November/December mice rarely engaged in fights if they had not received METH. Therefore, we only included mice from November/December in all the groups (METH/METH, Sal/METH, Sal/Sal, and intruders). We are therefore reporting data from two independent series of experiments carried out in 2001 and 2002, respectively. Mice for the experiments in which they received alternate single saline or single METH injections (see below) were born in June and displayed greater "basal" fighting activity compared with mice used to test the other groups (see above).

2.2. The duration of persistent sniffing/following at the initial encounter with an intruder

We noted that saline-treated "resident" mice consistently exhibit a characteristic behavior toward an intruder shortly after placing the intruder into the "resident" mice cage. The "resident" mouse approaches the intruder within 5–10 s after its placement and begins persistent sniffing of the head or genitals of the intruder. This is sometimes accompanied by following the intruder if the intruder tries to avoid contacts. Under conditions employed in the current study, this behavior in saline-treated mice typically continues for about 60 s. After this period of time, "host" mice usually discontinue sniffing/following but only occasionally renew it for several seconds. The duration of persistent sniffing of head or genitals accompanied by following/chasing was scored as the time in which the tested animal did not interrupt said behavior or stopped for less than 15 s.

2.3. Assessment of locomotor activity

The same Sal/Sal and METH/METH mice that were tested for aggressive behavior (Experiment 1) were also tested for locomotor activity. Tests were carried out 2 days before conducting tests for aggressive behaviors. Cages with mice were transported to the testing room 45–60 min before the start of the test session to decrease possible effects of stress associated with the transfer. Each animal was taken from its home cage, injected with either SAL (Sal/Sal group) or 6 mg/kg METH (METH/METH group), and returned to its home cage. Twenty hours later, the cage was placed into an activity monitor (Med. Associates, East Fairfield, VT). Mice were allowed to habituate to the activity monitor for 15 min and then activity was recorded for 15 min as cm/min traveled. For the METH/METH mice, activity was also measured 15 min after drug injection.

2.4. Statistical analyses

Differences in the fraction of animals initiating attack bite were assessed using chi-square tests for two groups and Kruskal–Wallis test for multiple groups. Differences in the latency before the first bite attack and in the duration of persistent sniffing/following between groups were examined using ANOVA followed by Tukey's post hoc test.

3. Results

3.1. Experiment 1: Fighting activity in mice after chronic or acute injections of METH

Fighting activity was measured in mice that received METH chronically (METH/METH group) and in mice that received a single dose of METH after chronic treatment with saline (Sal/METH). Both groups were compared with a control group of mice (Sal/Sal) that were chronically treated with saline. Fighting activity was measured 15 min and 20 h after the last injection. The 15-min time point was selected because mice chronically treated with METH displayed a clear increase in locomotor activity and stereotypic behaviors within 3–10 min after injection that continued for several hours (see below). The 20-h time point was selected because at this time, METH-induced increases in locomotor and stereotypic behaviors had subsided (see below).

Twelve percent (5 out of 42) of the Sal/Sal mice initiated a bite attack in this experiment (Fig. 1A). In contrast, 70% (21 out of 30) of the METH/METH group attacked an intruder when tested 15 min after METH, while 83% (20 out of 24) of these mice attacked an intruder when tested 20 h after drug administration. The difference between the METH/METH and Sal/Sal groups was highly significant ($\chi^2 = 10.84$, $P = .001$ for mice tested 15 min after injection and $\chi^2 = 76.80$, $P < .00001$ for mice tested 20 h after injection). On the other hand, mice that received a single dose of METH (Sal/METH group) showed no increases in the percentage of animals that attacked an intruder in comparison to the saline (Sal/Sal) group. Specifically, only 14% (2 out of 14) of these mice initiated a bite attack when tested 15 min after a single METH injection, while 25% (6 out of 24) of them attacked the intruder when tested 20 h after the single METH injection.

Latency before the first bite attack was significantly different between the treatment groups (ANOVA, $F = 11.78$, $df = 2,128$, $P < .0001$; Fig. 1B). The latency in the acute METH group (Sal/METH) was similar to that of the saline (Sal/Sal) group. In contrast, the latency in the chronic

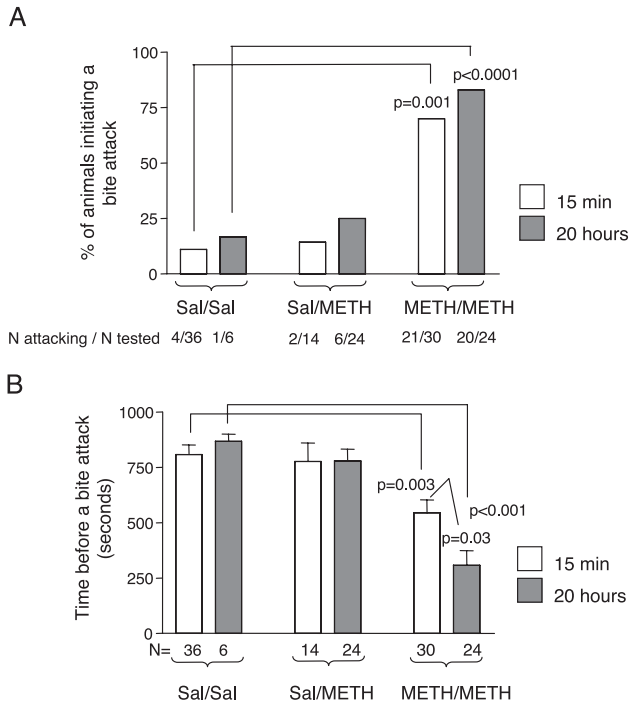

Fig. 1. Differences between groups of mice that received single or chronic doses of METH. Mice were either (i) chronically treated with saline (SalSal), (ii) chronically treated with saline and then received a single dose of METH (Sal/METH), or (iii) chronically treated with METH (METH/METH) as described in Methods. Behavioral tests were performed 15 min after injection or 20 h after injection. (A) Percentage of animals initiating a bite attack on intruder. Kruskal–Wallis test revealed significant differences between groups ($\chi^2 = 49.52$, $df = 5$, $P < .00001$). Chi-square test revealed significant differences between Sal/Sal and METH/METH mice (difference at 15 min after injection: $\chi^2 = 10.84$, $P = .001$; difference at 20 h after injection: $\chi^2 = 76.80$, $P < .00001$). (B) Latency before the first bite attack. One-way ANOVA revealed significant differences between the groups ($F = 11.78$, $df = 5,128$, $P < .0001$). Tukey's post hoc tests revealed significant ($P = .003$) differences between Sal/Sal and METH/METH mice 15 min after injection, significant ($P < .001$) differences between Sal/Sal and METH/METH mice 20 h after injection, and significant ($P = .03$) differences between the 15-min and 20-h time points in METH/METH mice.

METH group (METH/METH) was significantly shorter when compared to that of the Sal/Sal group ($P = .003$ for the 15-min test and $P < .001$ for the 20-h time point, Tukey's post hoc test). Interestingly, latency measured at 20 h after METH injection was shorter than that obtained at 15 min after the drug ($P = .03$, Tukey's post hoc test; Fig. 1B). These data indicate that it is chronic, not single injections, of METH that increase aggressiveness in mice.

3.2. Locomotor activity in mice chronically treated with METH

Locomotor activity in METH/METH mice measured at 20 h after injection was not different from that in Sal/Sal mice at 20 h after injection (264 ± 49 and 268 ± 30 cm/min traveled, respectively) but was increased at 15 min after injection (522 ± 168 cm/min traveled) (ANOVA, $F = 3.25$, $df = 2,43$, $P = .048$).

3.3. Experiment 2: Duration of persistent sniffing/following after the initial encounter with an intruder in mice chronically treated with METH (METH/METH)

We examined the duration of persistent sniffing after the initial encounter with an intruder because this behavior was considered to be a possible preamble to fighting. Six mice chronically treated with METH were alternately tested at 15 min and 20 h after injection (Fig. 2). As illustrated on Fig. 2, mice treated with METH chronically showed significant increases in the duration of continuous sniffing/chasing the intruder when tested 15 min after being injected with METH ($P < .004$, Tukey's post hoc tests). The same mice tested 20 h after METH showed a significantly shorter duration of sniffing compared to the 15-min time point ($P < .003$, Tukey's post hoc tests). These values were not different significantly from those of saline-treated mice (Fig. 2).

3.4. Experiment 3: Assessment of the effect of single alternating saline/METH injections on fighting activity and on the duration of persistent sniffing/following after the initial encounter with an intruder

As noted above (Experiment 1), comparison of Sal/Sal and Sal/METH groups revealed no significant effects of a single METH injection on fighting activity. Nevertheless, we decided to investigate the effect of a single METH injection by using a potentially more sensitive test. To accomplish this end, we used repeated tests of the same mice by alternating saline and METH injections. We reasoned that this paradigm might be less influenced by

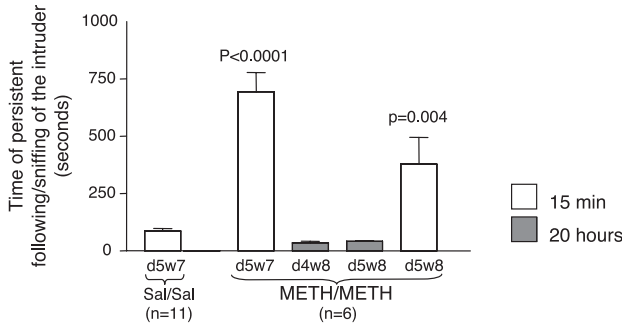

Fig. 2. Effects of time after injection on the duration of persistent sniffing/following during the initial encounter with an intruder in mice chronically treated with METH. Abbreviations: w#, week after beginning injections; d#, day of the week [e.g., d5w7 stands for Day 5 (Friday) of the 7th week after beginning injections]. ANOVA revealed significant differences among the groups ($F = 24.88$, $df = 4,34$, $P < .0001$). Tukey's post hoc test revealed that differences between METH/METH at 15 min after injection and Sal/Sal at 15 min after injection were significant and shown above bars ($P < .0001$ for the test at d5w7, and $P = .004$ for the test at d5w8). Differences between tests 15 min after METH and after 20 h after METH were all significant at $P < .003$ (not shown).

individual variations than comparison of different groups of mice treated with saline or METH. Two injection schedules, namely, Sal/METH/Sal/Sal and Sal/METH/Sal/METH, were used in an attempt to reduce possible confounds of learning, continuing isolation, and aging that might occur during the performance of these tests over time.

3.5. Fighting activity

Testing mice after alternating single saline or METH injections confirmed that there was no significant effect of single METH injection on fighting activity (Fig. 3A and B). Although the number of animals initiating a bite attack was nominally greater after METH injection in Week 2 than it was after saline in Week 1, it was not different from saline in Week 3. The number of attacks after the METH injection in Week 9 in the Sal/METH/Sal/METH group was even nominally lower than after saline in Week 3 (Fig. 3A). Moreover, there were no statistically significant differences between single saline and single METH injections in the number of animals initiating a bite attack in both the Sal/METH/Sal/Sal and Sal/METH/Sal/METH groups ($\chi^2 = 2.16$, $df = 3$, $P = .54$ and $\chi^2 = 5.98$, $df = 3$, $P = .11$, respectively, Kruskal–Wallis test). Similarly, there were no significant differences between single saline and single METH injections in the latency time before the first bite attack (Fig.

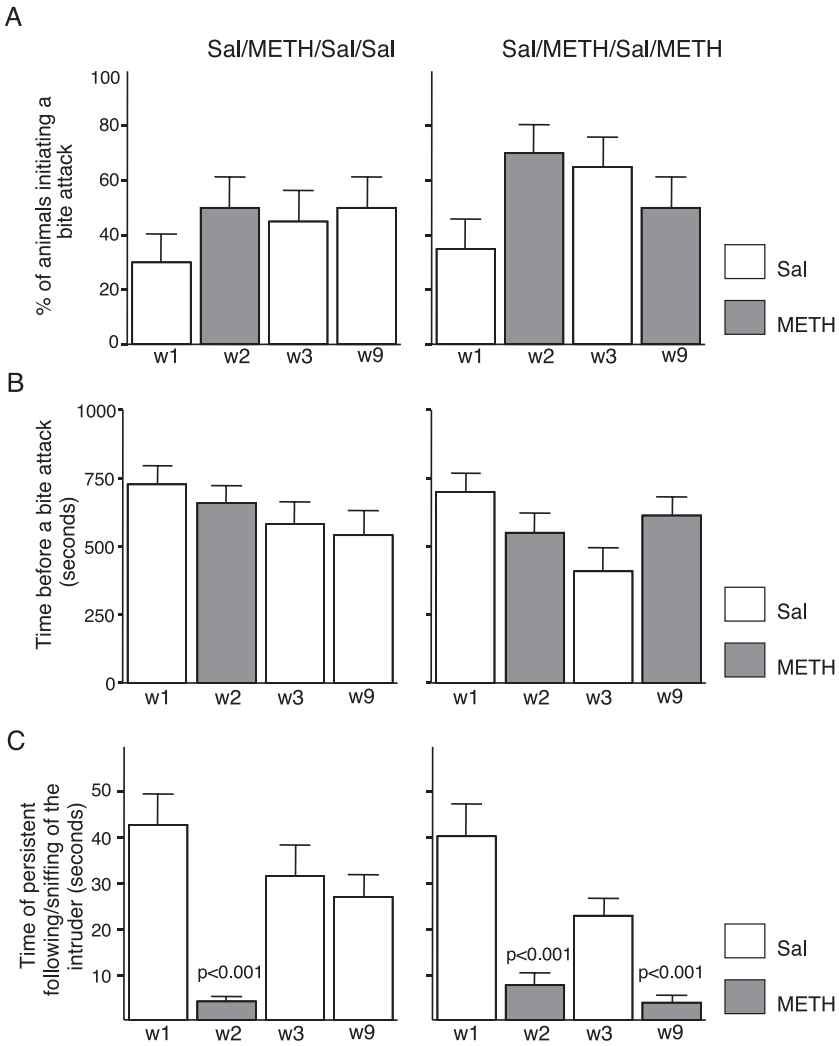

Fig. 3. Effects of a single dose of METH on fighting and the duration of persistent sniffing/following during the initial encounter with an intruder examined using alternating saline or METH injections. Forty 12- to 14-week-old male CD-1 mice were randomly assigned to two groups (Sal/METH/Sal/Sal and Sal/METH/Sal/METH, $n = 20$ in each group). Mice were single housed for 2 weeks. After this period of time (w1 on the graphs), mice in both groups received a saline injection and were tested. One week later, mice in both groups received a single dose of 6 mg/kg METH and were tested again (w2 on the graphs). After another 1 week, both groups received saline and were tested again (w3 on the graphs). Six weeks later, the Sal/METH/Sal/Sal group received saline while the Sal/METH/Sal/METH received 6 mg/kg METH and mice were tested again (w9 on the graph). In each series, mice were tested 15 min after injection. One-way ANOVA revealed significant differences between the treatments in the duration of persistent sniffing/following at the initial encounter with an intruder in the Sal/METH/Sal/Sal ($F = 9.49$, $df = 3,76$, $P < .001$) and Sal/METH/Sal/METH ($F = 14.91$, $df = 3,76$, $P < .001$) groups). Differences from the first saline injection are indicated above the bars (Tukey's post hoc tests).

3B). Specifically, ANOVA carried out for the Sal/METH/Sal/Sal group showed no significant changes ($F=1.12$, $df=3,76$, $P=.35$), whereas that run on the Sal/METH/Sal/METH group was only marginally significant ($F=2.73$, $df=3,76$, $P=.05$). Moreover, Tukey's post hoc tests showed no significant differences between Saline and METH treatments (all $P>.50$). These results from alternating single saline and single METH injections are consistent with the finding of no effect of single METH on fighting activity from the observations described under Experiment 1.

3.6. The duration of persistent sniffing/following after the initial encounter with an intruder

One-way ANOVA revealed significant differences between the treatments in the duration of persistent sniffing/following at the initial encounter with an intruder in the Sal/METH/Sal/Sal ($F=9.49$, $df=3,76$, $P<.001$) and Sal/METH/Sal/METH ($F=14.91$, $df=3,76$, $P<.001$) groups. There were significant decreases in the duration of sniffing/following after a single dose of METH ($P<.001$, Tukey's post hoc tests). The duration of this behavior reverted back to almost "basal" level 1 week later when mice were given saline (Fig. 3C). A single injection of METH given 6 weeks later (group Sal/METH/Sal/METH) again significantly reduced the duration of sniffing/following ($P<.001$, Tukey's post hoc tests). Injection of saline (Sal/METH/Sal/Sal group) did not show similar changes. These data indicate that single administration of METH may significantly and reversibly reduce the duration of initial sniffing/following of the intruder. This is different from the observations in mice treated chronically with METH; these mice show METH-induced increases in the duration of persistent sniffing/following at the initial encounter with an intruder measured at 15 min after an METH challenge (see Fig. 2). There was a trend toward a decrease in the duration of persistent following and sniffing of the intruder in both the Sal/METH/Sal/Sal and Sal/METH/Sal/METH groups during the 9-week period of observations (Fig. 3).

4. Discussion

Male CD-1 mice show low degree of aggressiveness under the conditions used in the present study. Therefore, they were useful in helping to investigate neuropharmacological effects of METH on the appearance aggressive behaviors. Quantitative tests conducted after 8 weeks of repeated METH injections demonstrated significant drug-induced increases in fighting activity in male CD-1 mice. These changes were evidenced by an increased number of animals initiating fights and by a reduction in the latency time before first bite attack. In contrast, no significant effects of single METH injections on these measures of aggressiveness were observed. Fighting activity in mice chronically treated with METH appears to be greater when measured at 20 h than at 15 min after an METH injection. Locomotor activity was similar to saline-treated mice at 20 h, thus providing evidence that increased fighting after chronic METH was not secondary to an overall increase in behavioral stimulation. There was also no association of aggressiveness with the duration of persistent sniffing/following after the initial encounter with an intruder. Our observations that fighting activity and persistent sniffing after the initial encounter with an intruder were differentially influenced by METH suggest that persistent sniffing at the initial encounter does not necessarily represents a preamble to fighting. These data also suggest that these two behavioral responses are secondary to METH-induced activation of different biochemical/physiological substrates in the brain.

METH abuse is known to be associated with violent behaviors by abusers (Carey and Mandel, 1968; Ellinwood, 1971a; Hawks et al., 1969; Szuster, 1990). Several studies have examined the effects of a single METH administration on aggressive behaviors in laboratory animals in stressful situations or in animals trained to fight by painful stimuli (Crowley, 1972; Maeda et al., 1985; Miczek and O'Donnell, 1978; Shintomi, 1975). For example, Shintomi (1975) reported that when seven mice injected with METH (5 mg/kg sc) were placed together into a box with a limited space, they demonstrated fighting behavior with squeaking. Maeda et al. (1985) also reported that METH administered intraperitoneally (0.5–3 mg/kg) lowered the thresholds for defensive directed attacks and hissing elicited by electrical stimulation of the VMH nucleus. Crowley (1972) reported the dose-dependent modulation of fighting time in pairs of rats that were first trained to regularly fight on an electric shock grid and both received METH ("electric foot-shock elicited defensive reaction"). At lower doses (0.25–1 mg/kg), METH stimulated fighting behavior and at higher doses (2 and 4 mg/kg) reduced it. In contrast, Miczek and O'Donnell (1978) reported no effect of single METH injections at doses below 8 mg/kg on the frequency of attacks and threat behavior by mice that were trained to fight prior the experiments. Because the inconsistency of the summarized data might reflect complex interactions among METH, stress, "learned" behaviors, pain responses, and aggressiveness, we used a paradigm that did not employ any painful stimuli and any prior training. It is important to note that our preliminary observations had shown that even mild stress, such as those associated with moving animals to another room, markedly enhanced fighting in mice (data not shown). To minimize the effects of moving the mice, we conducted the tests in the same room where animals were normally housed. Under these conditions, there were no significant effects of a single METH injection on fighting whereas the effects of chronic METH were highly significant.

Our observations also show that a single injection of METH caused differential effects on the duration of persistent sniffing of head or genitals of an intruder than those observed after chronic METH administration. A single dose

of METH reduced the duration of this behavior when measured 15 min after injection; these results are consistent with data on METH-induced social isolation in nonhuman primates (Crowley et al., 1974). In contrast, the duration of persistent sniffing of head or genitals was significantly increased in mice chronically treated with METH when measured 15 min after METH challenge. When taken together, these observations indicate that chronic administration of the drug might cause neuroadaptive changes that trigger the disinhibition of pathways for sniffing that are mediated by acute administration of METH to a naïve animal.

Although stereotypy was not quantitatively assessed in the current study, it is interesting to note that starting at Week 6, mice chronically treated with METH demonstrated marked stereotypic behaviors that varied across individual mice. The most common observed stereotypic behaviors were running in circles inside the cage, constant grooming of their heads, or poking their heads repeatedly in the same place(s). These behaviors become fully apparent 5–10 min after the METH injection and continued for several hours. No apparent stereotypy was evident 20 h after METH injection. These observations are consistent with those of other investigators who have reported intense stereotypic behaviors after chronic METH administration to rats (Kifune and Tadokoro, 1991), cats (Ellinwood and Escalante, 1970), and monkey (Ellinwood, 1971b).

Because of its tremendous social significance, normal and pathological degrees of aggressiveness have been intensively investigated using a variety of approaches; these include genetic, biochemical, and pharmacological approaches (Bell et al., 1999; Brodkin et al., 2002; Miczek et al., 2001; Miczek and O'Donnell, 1978). Although some important observations have been reported on regulatory pathways that influence aggressive behaviors, there still remains a substantial gap in our knowledge base. Several neurotransmitters, including serotonin, GABA, glutamate, opioids, cholecystokinin, substance P, norepinephrine, dopamine, and acetylcholine, have recently been implicated in the initiating, expression, and maintenance of aggressive behaviors (Brodkin et al., 2002; Chiavegatto et al., 2001; Olivier et al., 1995; Siegel et al., 1999). Because neuropharmacological actions of METH are known to involve interactions of multiple neurotransmitters, including serotonin, dopamine, GABA, glutamate, and some peptides (Bustamante et al., 2002; Chapman et al., 2001; Gibb et al., 1990; Hanson et al., 1991; Kokoshka et al., 1998), studies of these neurotransmitters in relationship to METH-induced aggressive behaviors in rodents will be a fruitful area of research. These studies should lead to a better understanding of the biochemical neuroanatomy of aggression. For example, in the rat, an area largely coincident with the intermediate hypothalamic area appears to be crucial for the expression of attacks (Siegel et al., 1999). In humans, components of the limbic–subcortical–mesencephalic continuum including the amygdala are thought to be prominent in the regulation and expression of aggression (Goldstein, 1974; Sachdev et al., 1992). The frontal lobes might play inhibitory controls because injuries to those brain regions can cause aggressive behaviors (Brower and Price, 2001).

In summary, we have observed significant increases in fighting behaviors by mice chronically exposed to METH using a drug regimen that mimics the escalating dosing that METH abusers tend to use. Increased fighting activity was not secondary to an overall increase in the level of METH-induced behavioral activation. It could be argued that the experiments, as presented in this paper, might not adequately address the question as to whether or not increased fighting is related to chronic METH because any difference observed after acute METH challenge could be due to difference in baseline fighting. Such an argument would miss the mark because any differences in baseline fighting between animals chronically treated with saline or METH injections would have also resulted from the administration of the active drug. This suggestion is supported by the fact that increased fighting was retained and was even greater at 20 h than at 15 min after METH injection because these observations also indicate the chronic injections of METH alter baseline fighting activity. The results are also consistent with the clinical literature, which has documented increased aggressiveness in chronic METH abusers (Carey and Mandel, 1968; Ellinwood, 1971a; Hawks et al., 1969; Szuster, 1990). Nevertheless, future studies will need to assess possible interactions between chronic exposures to METH, baseline fighting activity, and the time course of these behavioral changes. It will also be important to determine to what extent various METH regimens might affect aggressive behaviors in various environmental settings. Because of its potential clinical importance, it will be paramount to investigate the potential stability of METH-induced aggressiveness in mice during long intervals of drug abstinence. Finally, the molecular adaptations associated with various schedules of drug administration and their relationship to METH-induced aggressiveness will need to be elucidated.

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