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The Effects of High-Dose Methamphetamine in the Aging Rat: Differential Reinforcement of Low-Rate 72-s Schedule Behavior and Neurochemistry[1]

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ABSTRACT

High-dose methamphetamine (METH) causes damage to the dopamine and serotonin neurons in the brains of laboratory animals. The purpose of this report was to determine the long-term consequences of high-dose METH treatment on behavior and neurochemistry. Rats were trained on the differential reinforcement of low-rate 72-s (DRL 72-s) schedule of reinforcement. Twelve weeks after training began (age 23 weeks), they received one or three high-dose METH regimens. Each regimen consisted of four injections of 15 mg/kg, at 2-h intervals. Each regimen was separated by 7 weeks. A second group received METH treatment at age 23 weeks, but behavioral training was not initiated until the rats reached age 60 weeks. A third group received METH treatment without behavioral training. DRL behavior showed mild impairments 3 to 18 weeks after the onset of treatment; the impairments did not persist into middle age. At age 70 weeks, serotonin concentrations were decreased in somatosensory cortex, occipital cortex, and hippocampus but not in other subcortical structures. Serotonin tissue concentrations were enhanced in septum and striatum but only in rats receiving three regimens and behavioral training. Dopamine was not depleted at age 70 weeks. In three additional groups, one, two, or three METH regimens were administered, and tissue concentrations were measured 6 weeks after the last treatment (corresponding to the times of the behavioral test blocks in the DRL experiments). Serotonin depletions were noted in cortex, hippocampus, amygdala, and striatum but not in septum, hypothalamus, nucleus accumbens/olfactory tubercle, or ventral midbrain. Dopamine was decreased in striatum and septum but not in nucleus accumbens/olfactory tubercle, amygdala, hypothalamus, or ventral midbrain. DRL 72-s schedule impairments are attributed to serotonin depletions. Three METH regimens did not result in greater behavioral or neurochemical deficits than one regimen.

Humans who abuse the amphetamines maintain the habit for long periods of time (Williamson et al., 1997). Recently, Wilson et al. (1996) reported postmortem decreases in dopamine, tyrosine hydroxylase, and dopamine transporter levels (but not vesicular transporter) in individuals with a history of methamphetamine (METH) use. Similarly, McCann et al. (1998) found decreases in dopamine transporter density in vivo in abstinent METH users.

High doses of METH damage the dopamine and serotonin systems of the brain in laboratory animals. For example, dopamine and serotonin are depleted in multiple brain regions (Ricaurte et al., 1980; Richards et al., 1993a). Serotonin axon degeneration has been reported using immunocytochemistry (Axt and Molliver, 1991). Functional reuptake of dopamine and serotonin is impaired, and the activity of the synthetic enzymes tyrosine hydroxylase and tryptophan hydroxylase is diminished after high-dose METH treatment (Hotchkiss and Gibb, 1980; Ricaurte et al., 1980; Wagner et al., 1980). Several reports indicate that high-dose METH treatment has long-term consequences, depleting dopamine and serotonin tissue concentrations 6 to 8 months after treatment (Bittner et al., 1981; Friedman et al., 1998; Cass and Manning, 1999).

Behavioral deficits have been observed after high-dose METH treatment in a reaction time task up to 3 months post-treatment (Richards et al., 1993a) and in a balance beam task 1 month post-treatment (Walsh and Wagner, 1992). Friedman et al. (1998) reported a temporary behavioral deficit on the Morris water maze after high-dose METH treatment. In another report, behavioral changes were not detected 2 weeks after METH treatment in food and water intake, performance on a fixed consecutive number task, avoidance responding, and open field activity (Seiden et al., 1993).

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ABBREVIATIONS: METH, methamphetamine; 6-OHDA, 6-hydroxydopamine; IRT, interresponse time; PkA, peak area; PkL, peak location; SAL, saline; DRL, differential reinforcement of low-rate; 5,7-DHT, 5,7-dihydroxytryptamine.

In this report, we studied the effects of high-dose METH treatment on behavior and neurochemistry during the aging process. The behavioral task we used was the differential reinforcement of low-rate 72-s (DRL 72-s) schedule. The DRL schedule is sensitive to the effects of the aging process (Soffie and LeJeune, 1991) as well as serotonin depletion caused by the selective neurotoxin 5,7-dihydroxytryptamine (5,7-DHT; Wogar et al., 1992, 1993; Fletcher, 1995; Jolly et al., 1999). The DRL impairments reported by Jolly et al. (1999) included increased response rate, decreased reinforcement rate, and large changes in the distribution of waiting times generated by the DRL 72-s schedule. In lesioned rats, the peak of the waiting time distribution was shifted toward shorter durations, and the distribution of waiting times was more variable [see Richards and Seiden (1991) and Richards et al. (1993b) for a discussion of DRL schedule behavior analysis]. The 5,7-DHT-induced lesions used in all of the DRL studies discussed above resulted in greater than 90% depletion of serotonin throughout the forebrain.

The first purpose of the experiments described here was to compare the effects on behavior and neurochemistry of a single high-dose METH regimen and three high-dose METH regimens (separated by 7 weeks). Because humans who abuse METH do not limit themselves to a single exposure, this multiple-exposure manipulation was meant to closer approximate a human abuse pattern compared with the single-regimen exposure. Our second purpose was to study the effects of METH treatment throughout the young-adult and middle-age time periods of the life of a rat.

It was hypothesized that high-dose METH exposure during young adulthood would cause dopamine and serotonin depletions in the aging rat; rats receiving three METH regimens would show greater depletions than rats receiving one METH regimen. Based on the DRL deficits observed after 5,7-DHT-induced serotonin depletions, it was hypothesized that high-dose METH treatment would impair DRL 72-s schedule behavior. This behavioral impairment would be seen during young adulthood and middle age.

Materials and Methods

Five experiments were conducted to study the effects of multiple METH regimens on the aging process. In the first experiment, initial training on the DRL 72-s schedule occurred before METH exposure. These rats were tested periodically during a 14-week treatment period, as well as after treatment had ended, up to 70 weeks of age. The treatment period included one or three METH regimens; each regimen consisted of four injections, regimens were separated from each other by 7 weeks. In the second experiment, rats received METH according to the same schedule as experiment 1, but training on the DRL 72-s schedule did not occur until after METH treatments had ended; training was not initiated until the animals were in middle age. In the third experiment, rats were given the same METH treatments used in experiments 1 and 2. However, they received no behavioral training (see Table 1). In the fourth experiment, rats received one, two, or three METH regimens and no behavioral training. They were sacrificed at times corresponding to specific points in the behavioral testing of rats in experiment 1 (see Table 2). The fifth experiment was included to study the degree of dopamine and serotonin depletions at an early time point (2 weeks) after a single METH regimen.

Animals

Male Sprague-Dawley rats (Harlan) weighing between 250 and 275 g (8 weeks old at the start of the experiment) were used. The rats were housed two per cage in hanging stainless steel wire cages. Lights were on in the colony room from 7:00 AM to 7:00 PM. Food was available freely. Access to water was restricted to 20 min/day. On training days, the rats received 20-min access to water at the end of their training session. On nontraining days (weekends), the rats were given 20-min access to water between 10:00 AM and 2:00 PM.

Drug Treatment

(+)-Methamphetamine hydrochloride (Sigma Chemical Co., St. Louis, MO) was dissolved in saline to form an injectable solution of 1 ml/kg. The dose used was 15 mg/kg/injection (calculated as the salt), administered four times per regimen, at 2-h intervals. Control rats received four injections of saline, at 2-h intervals per regimen. Depending on the experiment, regimens were administered one, two, or three times, with 7 weeks between intervals.

Before METH treatment, temperature-sensitive transmitters (Mini-Mitter model VM-FH) were implanted into the abdominal cavity. The rats were anesthetized with 7 mg/kg xylazine and 100 mg/kg ketamine. One week later, the rats received their first METH regimen. For the entire regimen, up to a period of 24 h after the first METH injection, rats were housed in environmental chambers. A computer monitored their body temperature and controlled the ambient temperature of the chambers. If a rat's body temperature reached 39.5°C, a cooling unit was activated to lower the core body temperature below 39.5°C. At all other times the chamber temperature was maintained at 24 (± 0.5)°C through activation of heating or cooling units.

Postmortem Tissue Assay

At the completion of the study, the rats were sacrificed, their brains were removed, and 10 different regions were dissected over ice. Regions that were dissected according the procedure of Heffner et al. (1980) were the frontal cortex, somatosensory cortex, occipital cortex, hippocampus, amygdala, nucleus accumbens/olfactory tubercle, striatum, septum, hypothalamus, and ventral midbrain. These 10 regions were chosen for tissue analysis because they are thought to be representative of the dopamine and serotonin systems of the brain. Serotonin is seen throughout the brain. Dopamine is seen in its highest concentrations in striatum and nucleus accumbens/olfactory tubercle. Tissue concentrations of dopamine and serotonin were measured using reversed phase HPLC-EC. Instruments and chromatographic conditions included an HPLC pump (LC10-AD; Shimadzu, Kyoto, Japan), pulse dampener (model LP-21; SSI, purchased from Alltech Associates, Deerfield, IL), injection valve (model 7010; Rheodyne, Cotati, CA); coulometric detector (model 5200; ESA, Chelmsford, MA) with applied voltages of −200 and +400 mV, and column (100 × 4.6 mm, Adsorbosphere; Alltech Associates: catecholamine, C18 resin, 3-μm particle size). Analyses were performed with EZ-Chrome Chromatography Data System, version 6.7 (purchased from ESA). Mobile phase consisted of 0.15 M monochloroacetic acid, 2 mM EDTA, 200 mg/l octyl sulfonic acid, 0.1325 M NaOH, 1.4% acetonitrile, and 0.8% tetrahydrofuran, pH 3.

Experiment 1: DRL Performance

Apparatus. The rats were tested in operant chambers (20.5 × 20.5 × 23.5 cm) with grid floors, aluminum front and back walls, and Plexiglas sides. A lever was mounted on the front panel 3 cm above the grid floor and 4.5 cm from the nearest side. A downward force of approximately 0.15 N was required to depress the lever. A solenoid-operated dipper was located 10 cm to the left of the lever; access to the dipper was through a round 4.5-cm-diameter hole in the front panel. Reinforcement consisted of lifting the dipper (0.025 ml) from a water trough to within reach of the rat's tongue for a period of 4 s. A house light, mounted 15 cm above the floor on

TABLE 1

Age of rats at different time points in experiments 1, 2, and 3.

The animals were treated at age 23, 30, and 37 weeks. These ages were chosen for the following reasons: the 23-week age used for the initial treatment allowed for a 12-week training period before METH treatment. The 7-week interval used between the three regimens allowed for a 3-week post-treatment recovery period, and a 3-week test period after each treatment regimen. The 7th week of the interval was used to return the rats to free access drinking 1 week before treatment. The 20 weeks of no DRL training (experiment 1) allowed the rats to reach middle age for further testing.

Age	Treatment	Performance (Expt. 1)	*n*	Acquisition (Expt. 2)	*n*	No Training (Expt. 3)	*n*
wk							
8		Rats arrive	Sal = 24 1× = 20 3× = 28	Rats arrive	Sal = 24 1× = 20 3× = 28	Rats arrive	Sal = 18 1× = 14 3× = 16
10		Begin DRL (12 wk)					
22		Transmitter implant		Transmitter implant		Transmitter implant	
23	METH regimen 1						
26		Resume DRL (3 wk)	Sal = 24 1× = 19 3× = 24				
30	METH regimen 2						
33		Resume DRL (3 wk)	Sal = 24 1× = 19 3× = 24				
37	METH regimen 3						
40		Resume DRL	Sal = 24 1× = 19 3× = 24				
43		No DRL testing (20 wk)					
60				Begin acquisition (10 wk)	Sal = 8 1× = 16 3× = 11		
62		Resume DRL (6 wk)	Sal = 17 1× = 10 3× = 9				
70		End experiment; tissue assays	Sal = 17 1× = 10 3× = 9	End experiment; tissue assays	Sal = 4 1× = 13 3× = 9	End experiment; tissue assays	Sal = 16 1× = 13 3× = 12

TABLE 2

Age of rats relative to sequence of events in experiment 4

Rats received one, two, or three regimens of METH (each regimen consisted of 15 mg/kg/injection, four injections, 2-h intervals). Regimens were separated by 7 weeks. The animals were treated at age 23, 30, and 37 weeks. These ages were chosen to parallel the treatment ages in experiments 1 and 2. The initial time point and the 7-week intervals between regimens allowed for DRL 72-s schedule training and testing before and between METH regimens in the preceding experiments.

Age of Rat	METH 3-REG	*n*	METH 2-REG	*n*	METH 1-REG	*n*
wk						
8	Rats arrive	SAL = 12 METH = 12	Rats arrive	SAL = 12 METH = 17	Rats arrive	SAL = 13 METH = 15
22	Transmitter implant		Transmitter implant		Transmitter implant	
23	METH regimen 1		METH regimen 1		METH regimen 1	
29					End experiment	SAL = 13 METH = 15
30	METH regimen 2		METH regimen 2			
37	METH regimen 3		End experiment	SAL = 12 METH = 17		
43	End experiment	SAL = 10 METH = 12				

the back wall, was turned on when a training session began and off when the training session ended. The operant chambers were connected to a PDP-11/73 microcomputer via a Coulbourn Lablinc interface (Coulbourn Instruments, Allentown, PA). The schedule contingencies were programmed using the SKED-11 software system (Snapper et al., 1976). The timing resolution of the system was 0.01 s.

Behavioral Training. On arrival in the colony, the rats were adapted to the 20 min/day access to water regimen for 1 week. They were then trained to bar press in overnight training sessions using an alternative fixed-ratio 1 (FR1), fixed time 1-min schedule. Overnight training occurred until the rats reached a criterion of two consecutive nights with 100 bar presses. Rats that did not acquire the lever press response after five overnight training sessions were hand shaped. Once the rats acquired the lever press response, they were shifted to daily, 1-h training sessions on the DRL 72-s schedule (5 days a week).

Interresponse Time (IRT) Analysis. On a DRL 72-s schedule of reinforcement, the rats are reinforced for waiting at least 72 s be-

tween each response. The IRT distributions of rats trained on DRL schedules are frequently bimodal, with one mode occurring at very short IRT durations and a second mode occurring at longer IRT durations. The longer duration IRT distributions generated by DRL 72-s schedule performances were quantitatively characterized using peak deviation analysis. Peak deviation analysis includes two measures for the characterization of the profile of DRL IRT distributions: peak area (PkA) and peak location (PkL) (Richards and Seiden, 1991; Richards et al., 1993b).

Peak deviation analysis compared the obtained IRT distribution of each rat with a theoretical distribution that predicts the appearance of the obtained IRT distribution had the rat emitted responses at the same overall rate but randomly in time with respect to the preceding response. This expected random curve is called the corresponding negative exponential and is based on the mean of the obtained pause IRT durations with bursts (IRTs < 6 s) excluded (Richards and Seiden, 1991; Richards et al., 1993b).

The PkA is the area under the curve of the IRT distribution. The PkA is the proportion of the obtained pause IRTs above the corresponding negative exponential. The PkL represents the center of the IRT distribution. It is the central IRT duration (median) of the area above the corresponding negative exponential.

Procedure. Rats in the performance experiment arrived in the colony at 8 weeks of age. One week after arrival, they were placed on restricted access to water (20 min/day); 1 week later, DRL 72-s training began. Training continued for 12 weeks, after which the rats received their first METH regimen (METH 3-REG group, $n = 28$), their only METH regimen (METH 1-REG group, $n = 20$), or saline (SAL group, $n = 24$). After each regimen, a 3-week time-off period (no training) was followed by 3 weeks of training. One week before each regimen, rats were given free access to water; water restriction was reinstated 2 weeks after the regimen. Training ceased for a 20-week period after regimen 3; training was then reinstated for 6 additional weeks. Animals were sacrificed at the completion of this 6-week test period, at age 70 weeks.

Table 1 describes the sequence of events that occurred in this experiment. The different temporal intervals outlined in this table were chosen for the following reasons. METH treatment first occurred at age 23 weeks to allow rats in the DRL performance experiment to have sufficient time (12 weeks) to be trained to stability on the DRL schedule before receiving METH. The 7-week interregimen interval was chosen to allow 1) a recovery period (3 weeks) after the METH regimen, 2) a test period (3 weeks) sufficiently long to allow for accurate behavioral assessment after each METH regimen, and 3) 1 week of free access to water before METH treatment. After a 20-week test-free period, DRL 72-s schedule training resumed at age 62 weeks. Reinstatement of training at this time point allowed us to determine whether exposure to METH during early-adulthood affected the retention of a previously learned operant schedule in middle age.

Data Analysis: Behavioral Measures. Response rate, reinforcement rate, and PkA and PkL measures on the DRL 72-s schedule were determined for each rat. The data were averaged for each rat across each 3-week post-METH test period. For the final 6-week test period, only the last 3 weeks were analyzed; data were averaged for each rat across this 3-week period. A number of animals were lost between age 43 weeks and age 62 weeks (see Table 1). The last test period (age 62 weeks) was therefore analyzed separately. For baseline and testing at age 26, 33, and 40 weeks, a two-factor ANOVA was used (factors: treatment and time). When significant effects were identified with the ANOVA, subsequent analysis was performed using the Newman-Keuls post hoc test (Statistica software). One-factor ANOVAs were used to analyze the fourth behavioral block (age 62 weeks).

Data Analysis: Neurochemical Measures. Statistical analyses of dopamine and serotonin tissue concentrations for experiments 1, 2, and 3 were performed together. (Note that tissue sample assays from rats in experiments 1, 2, and 3 were performed at different times.) For each neurotransmitter and each brain region, a two-factor ANOVA was used. The factors were treatment (SAL, METH 1-REG, and METH 3-REG) and training condition (no training, acquisition, and performance). Post hoc analysis was performed using the Newman-Keuls test. Bonferroni corrections were applied to allow for comparisons in multiple brain regions (see *Results*).

Experiment 2: Acquisition

To evaluate the effects of early METH exposure (during young adulthood) on the ability to learn during middle age, DRL 72-s schedule acquisition training began at age 60 weeks.

All methods were the same as experiment 1 (performance) except for the procedural time line (see Table 1). The rats arrived in the colony at 8 weeks of age and received their first METH regimen (METH 3-REG group, $n = 28$), their only METH regimen (METH 1-REG, group, $n = 20$), or saline (SAL, $n = 24$) at age 23 weeks. METH treatments were administered and water access was regulated during the 14-week treatment period as described in experiment 1. Rats were maintained on the same watering schedule, even though DRL 72-s schedule training had not begun, to ensure similarity between experiments 1 and 2. At age 60 weeks, DRL 72-s schedule acquisition training began according to the methods described in experiment 1. The rats were trained for 10 weeks and then sacrificed at age 70 weeks. See Table 1 for the sequence of events in experiment 2.

Data Analysis: Behavioral Measures. Two-factor ANOVAs were used to analyze PkA, responses, reinforcers, and PkL. The factors were treatment and time.

Data Analysis: Neurochemical Measures. Dopamine and serotonin tissue concentrations were analyzed as described in experiment 1.

Experiment 3: No Behavioral Training I

The purpose of this experiment was to have a "no training" comparison group for the neurochemical analysis of rats in experiments 1 and 2. The rats were housed in a different facility but were maintained under the same housing, water restriction, and treatment conditions as described in experiment 1; they received no behavioral training.

Data Analysis: Neurochemical Measures. Dopamine and serotonin tissue concentrations were analyzed as described in experiment 1.

Experiment 4: No Behavioral Training II

The goal of experiment 4 was to determine the effects of METH treatment on tissue concentrations at specific time points relative to behavioral training. Rats were treated with one, two, or three METH regimens, beginning at age 23 weeks, and sacrificed 6 weeks after their last treatment. Methods were the same as described earlier except for the following.

The rats were housed in a different facility but were maintained under the same housing, water restriction, and treatment conditions as described in experiment 1. Three groups of rats were used. The METH 3-REG group received three regimens at 7-week intervals and was sacrificed at age 43 weeks, 6 weeks after the third regimen ($n = 12$ METH, 12 SAL). The METH 2-REG group received two regimens at a 7-week interval and was sacrificed at age 37 weeks, 6 weeks after the second regimen ($n = 17$ METH, 12 SAL). The METH 1-REG group received one regimen and was sacrificed at age 29 weeks, 6 weeks after the single regimen ($n = 15$ METH, 13 SAL). See Table 2 for the sequence of events.

Data Analysis: Neurochemical Measure. Two-factor ANOVAs were used to analyze dopamine and serotonin tissue concentrations. The factors were treatment (saline or METH) and number of regimens (one, two, or three). Post hoc analysis was performed using the Newman-Keuls test. To allow for comparisons made in multiple

brain regions, the Bonferroni correction was used for each neurotransmitter system (see *Results*).

Experiment 5: Short-Term Effects of a Single METH Regimen

The final experiment was designed to determine the short-term effects of a single METH regimen on dopamine and serotonin tissue concentrations. The rats were housed one per cage in hanging stainless steel wire cages. Food and water were freely available. The rats were sacrificed 2 weeks after a single METH regimen (15 mg/kg/injection, four times, 2-h intervals, $n = 5$) or saline treatment ($n = 6$). Although the housing and feeding conditions differed from the previous experiments in this report, the METH treatment conditions (dose, injection interval, temperature control) were the same. The effects of METH treatment on dopamine and serotonin in this experiment allowed us to approximate the degree of depletion experienced 2 weeks post-treatment by the rats of experiments 1 to 4, which had longer survival times.

Data Analysis: Neurochemical Measures. Two-factor ANOVAs (brain region and treatment) were used to analyze dopamine and serotonin tissue concentrations. Post hoc analysis was performed using the Newman-Keuls test.

Results

Experiment 1: DRL 72-s Schedule Performance

After 12 weeks of DRL 72-s schedule training rats received one METH regimen or three METH regimens, separated by 7 weeks. Behavior was tested after each regimen period regardless of whether the rats were in the METH 1-REG or METH 3-REG groups. All behavioral measures are presented in Fig. 1.

PkA. The results of a two-factor ANOVA indicated a significant effect of time ($F_{3,189} = 8.45$, $P = .00003$) and a significant interaction between treatment and time ($F_{6,189} = 4.66$, $P = .0002$). Post hoc analysis indicated a significant decrease in PkA in the METH 1-REG group after the first and third regimen periods (first and third behavioral blocks, Fig. 1) relative to saline-treated rats and a decrease in the METH 3-REG group after the third regimen period.

Responses. The results of a two-factor ANOVA showed a significant effect of time ($F_{3,189} = 4.56$, $P = .0041$) and a significant interaction between treatment and time ($F_{6,189} = 3.29$, $P = .0042$). Post hoc analysis indicated a significant increase in responses in the METH 1-REG group in the first behavioral block.

Reinforcers. The results of a two-factor ANOVA showed a significant effect of time ($F_{3,189} = 6.243$, $P = .0005$) and a significant treatment × time interaction ($F_{6,189} = 3.29$, $P = .0042$). Post hoc analysis failed to uncover group differences within each time interval.

PkL. The results of a two-factor ANOVA showed a significant effect of time ($F_{3,189} = 7.15$, $P = .0001$) and a significant interaction ($F_{6,189} = 2.15$, $P = .05$). Post hoc analysis failed to uncover group differences within each time interval.

Behavioral Effects in Middle Age. There were no effects of high-dose METH treatment when the animals were tested again at age 62 weeks (fourth behavioral block, Fig. 1).

Experiment 2: DRL 72-s Schedule Acquisition at 60 Weeks of Age

There were no significant changes in the rats' behavior as a result of METH pretreatment when DRL 72-s acquisition was begun 37 weeks after the single METH regimen (METH

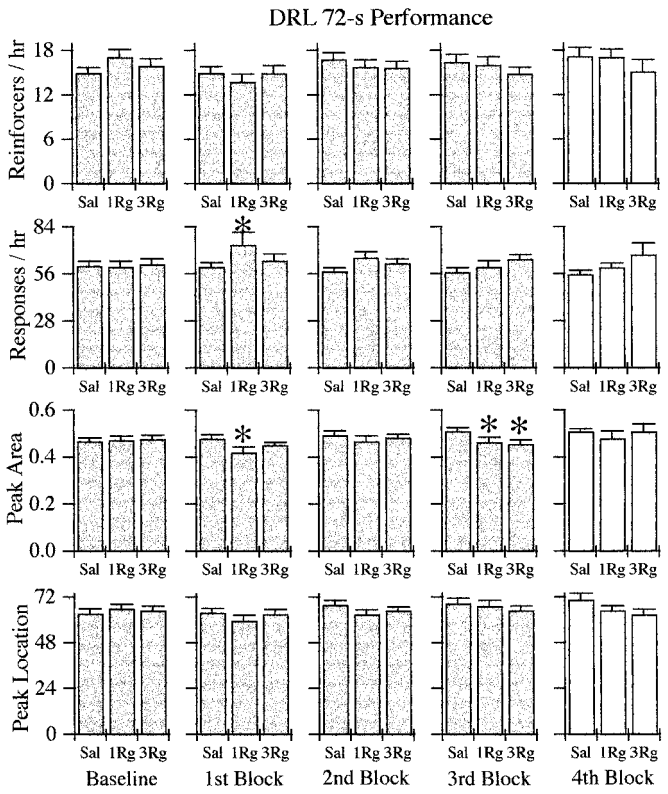

Fig. 1. Effects of high-dose METH on the performance of DRL 72-s schedule behavior. Animals were trained for 12 weeks before receiving METH treatment. Rats in the 1-REG (1Rg) group received one regimen of METH (15 mg/kg/injection × 4, at 2-h intervals). Rats in the 3-REG (3Rg) group received three regimens; each regimen was separated by 7 weeks. Baseline represents data collected during the 3 weeks before treatment (age 19–21 weeks). The first block represents data collected after the first regimen (age 26–28 weeks). The second block represents data collected after the second regimen (or the equivalent time period in saline or 1Rg rats, age 33–35 weeks). The third block represents data collected after the third regimen (age 40–42 weeks). *, significantly different from saline (Sal). Data from the fourth testing block (open columns) were analyzed separately due to a loss of subjects between week 43 (end of block 3) and week 62 (beginning of block 4).

1-REG group) or 23 weeks after the third METH regimen (METH 3-REG group), in comparison with a saline control group (SAL group). Figure 2 shows the results for PkA, PkL, responses, and reinforcers.

There was a significant effect of time for response rate ($F_{3,87} = 3.763$, $P = .014$), PkA ($F_{3,87} = 23.647$, $P < .001$), and PkL ($F_{3,87} = 5.874$, $P = .001$), demonstrating learning in rats pretreated with METH and SAL when trained at 60 weeks of age.

Experiments 1, 2, and 3: Neurochemical Measures

Rats were treated with either one or three high-dose METH regimens. In addition, they received either no behavioral training (no-training group), DRL performance training (performance group), or DRL acquisition training in middle age (acquisition group). The sample size for the two behavioral training groups dropped substantially over the course of the experiment; a similar loss of subjects was not seen in the no-training group. The rats in the behavioral groups were housed in a different facility than the rats in the no-training group. Although the cause for differential mortality rates is not known, the METH regimen itself and factors resulting from water restriction can be ruled out, because all three

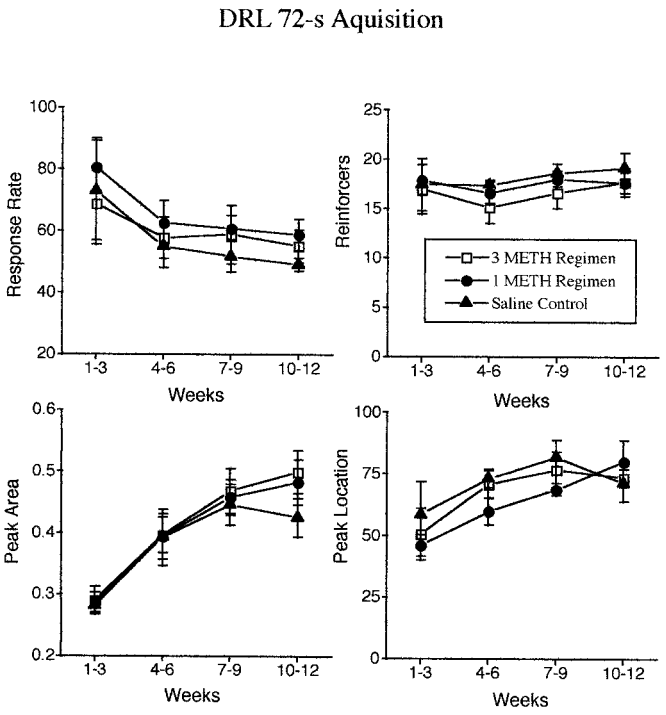

Fig. 2. Effects of high-dose METH pretreatment on the acquisition of DRL 72-s schedule behavior in middle age. Rats were treated with either one regimen or three regimens (at 7-week intervals), beginning at age 23 weeks; these rats received their first behavioral training at age 60 weeks. Each regimen consisted of 15 mg/kg/injection, four injections, at 2-h intervals. There was no effect of METH pretreatment.

groups experienced the same METH treatment and water access schedules.

Dopamine. Two-factor (treatment and training condition) ANOVAs identified the following main and interaction effects. Amygdala had a significant main effect of training condition ($F_{2,90}$ = 8.65, P = .0004). Nucleus accumbens/olfactory tubercle showed no significant effects. Striatum had significant main effects of treatment ($F_{2,91}$ = 4.65, P = .012) and training condition ($F_{2,91}$ = 134.95, P < .00001). Septum had a significant effect of treatment ($F_{2,91}$ = 5.89, P = .0039), training condition ($F_{2,91}$ = 42.16, P < .00001), and treatment × training condition interaction ($F_{4,91}$ = 4.02, P = .0048). Hypothalamus had significant treatment ($F_{2,90}$ = 5.41, P = .006) and training condition ($F_{2,90}$ = 94.33, P < .00001) effects. Ventral midbrain had significant training condition ($F_{2,92}$ = 76.39, P < .00001) and treatment × training condition interaction ($F_{4,92}$ = 4.30, P = .0031) effects.

Post hoc analyses were performed using the Newman-Keuls test; because comparisons were made in each brain region, a Bonferroni correction was applied. For the main effect of training condition, rats in the acquisition group showed decreased amygdala dopamine compared with the performance group. In the striatum and septum, all three training conditions resulted in different dopamine concentrations. For septum, the order of this difference was no-training group < acquisition group < performance group; for striatum, the no-training group had the lowest dopamine levels and the acquisition group had the highest levels. In the hypothalamus, the no-training and acquisition groups both had lower dopamine levels than the performance group. In the ventral midbrain, the no-training group had lower dopamine levels than the acquisition and performance groups (see Fig. 3).

For the main effect of treatment, post hoc analysis failed to uncover significant differences in striatum and hypothalamus. Only one interaction effect resulted in a significant pairwise comparison with post hoc analysis. Septal dopamine in the performance group was *enhanced* relative to saline-treated rats.

Serotonin. Two-factor (treatment and training condition) ANOVAs identified the following significant effects. Somatosensory cortex had significant main effects of training condition ($F_{2,93}$ = 8.96, P = .00028) and treatment ($F_{2,93}$ = 16.60, P = .000001), with no interaction. Occipital cortex (F2,88 = 13.71, P = .000007) and hippocampus ($F_{2,88}$ = 20.94, P < .000001) had significant main effects of treatment. Amygdala had a significant main effect of training condition ($F_{2,90}$ = 5.62, P = .005). Frontal cortex ($F_{4,93}$ = 3.161, P = .0175), nucleus accumbens/olfactory tubercle ($F_{4,88}$ = 8.30, P = .00001), hypothalamus ($F_{4,91}$ = 2.60, P = .041), striatum ($F_{4,92}$ = 6.161, P = .000195), septum ($F_{4,92}$ = 10.0, P = .000001), and ventral midbrain ($F_{4,93}$ = 9.259, P = .000002) all had significant interaction effects.

Post hoc comparisons were performed on treatment marginal means for brain regions showing no interaction effects. Post hoc pairwise comparisons were made for brain regions showing significant interaction effects. A Bonferroni correction was applied. Post hoc analysis of marginal means for treatment indicated a METH-induced decrease in serotonin for somatosensory cortex, occipital cortex, and hippocampus. The interaction effect identified for the striatum was due to an enhancement of serotonin in the acquisition rats treated with three METH regimens. The interaction effect identified for the septum was due to an enhancement of serotonin in the performance rats treated with three METH regimens. No other pairwise comparisons in brain regions showing interaction effects met the Bonferroni correction criterion (see Fig. 4).

Experiment 4: Neurochemical Measures

Rats were treated with one, two, or three METH regimens and sacrificed 6 weeks after the last regimen.

Dopamine. Two-factor ANOVAs (treatment and number of regimens) identified the following effects. In striatum ($F_{1,64}$ = 15.88, P = .00018) and septum ($F_{1,63}$ = 7.07, P = .0099), there were significant main effects of treatment. Post hoc analysis on marginal means confirmed the differences between saline and METH when collapsed across number of regimens. In the nucleus accumbens/olfactory tubercle, a significant main effect of number of regimens was identified ($F_{2,67}$ = 3.87, P = .0257). Post hoc analysis failed to identify a significant difference between marginal means. Amygdala, hypothalamus, and ventral midbrain showed no METH-induced changes in dopamine (see Fig. 5).

Serotonin. We conducted two-factor ANOVAs (treatment and number of regimens) within each brain region. For frontal cortex ($F_{1,66}$ = 12.68. P = .0007), somatosensory cortex ($F_{1,72}$ = 39.33, P < .00001), occipital cortex ($F_{1,67}$ = 33.69, P < .00001), hippocampus ($F_{1,64}$ = 78.6544, P < .00001), amygdala ($F_{1,64}$ = 43.64, P < .00001), and striatum ($F_{1,64}$ = 12.07, P = .00092), significant main effects of treatment were identified. For somatosensory cortex ($F_{2,72}$ = 5.226, P = .0076), occipital cortex ($F_{2,67}$ = 14.59, P < .00001), hippocam-

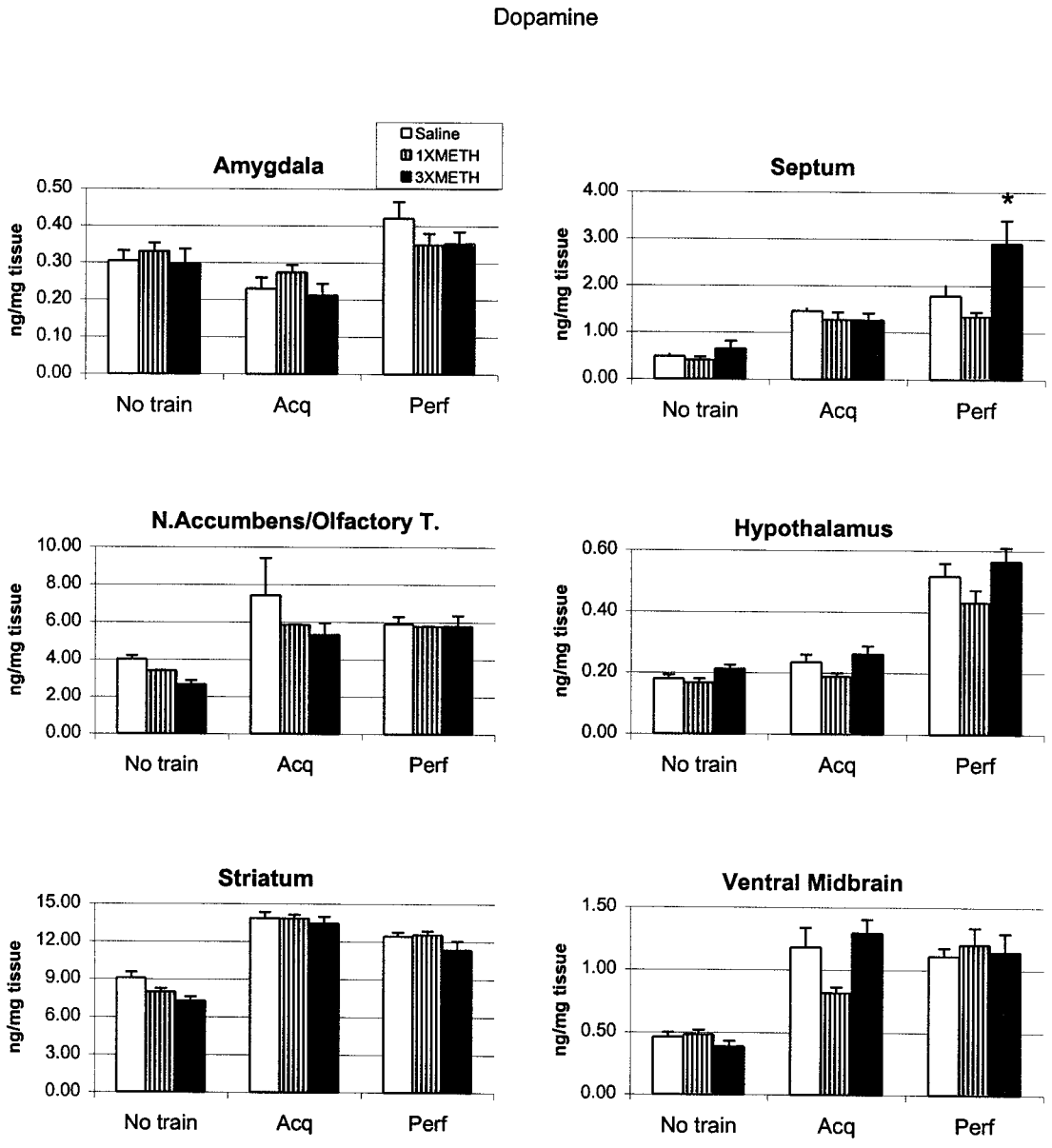

Fig. 3. Effects of high-dose METH pretreatment on postmortem dopamine tissue concentrations. Each regimen consisted of 15 mg/kg/injection, four injections, at 2-h intervals. Animals were sacrificed 33 weeks (3×METH, three-regimen groups) or 47 weeks (1×METH, one-regimen groups) after the last exposure to METH (age 70 weeks). Regimens were separated by 7 weeks. *, significantly different from saline. No train indicates the rats received no DRL training; acq, DRL acquisition at age 60 weeks; perf, DRL performance. Initial training in the performance group occurred before METH treatments began.

pus ($F_{2,64}$ = 5.342, P = .0072), septum ($F_{2,63}$ = 29.98, P < .00001), and striatum ($F_{2,64}$ = 3.832, P = .0268), main effects of number of regimens were identified. Hypothalamus ($F_{2,64}$ = 6.356, P = .003), nucleus accumbens/olfactory tubercle ($F_{2,67}$ = 5.2059, P = .0079), and ventral midbrain ($F_{2,64}$ = 6.28, P = .00322) had significant treatment × number of regimen interactions.

Post hoc comparisons were performed on treatment marginal means for brain regions showing no interaction effects; pairwise comparisons were made for brain regions showing significant interaction effects. A Bonferroni correction was applied. Frontal cortex, somatosensory cortex, occipital cortex, hippocampus, amygdala, and striatum showed significant METH-induced decreases in serotonin (marginal means). Post hoc analysis of interaction effects showed no significant decreases in serotonin (see Fig. 6).

Temperature. Figure 7 shows the average core temperature for each group, during each regimen, across the treatment period (first 1000 min, i.e., 16 h). From these values, it can be seen that individual injections of 15 mg/kg METH resulted in increases in core body temperature. This pattern is apparent throughout individual regimens, as well as in all cases of multiple regimens (groups METH 1-REG, METH 2-REG, and METH 3-REG). Table 3 shows the maximum temperature attained during the 24-h treatment period for each regimen.

Correlations were performed between maximum core temperature and tissue concentrations in each brain region listed below. Each METH treatment group was analyzed separately. Maximum core temperature was the highest temperature each rat attained during METH treatment. For rats that received more than one regimen, the highest tempera-

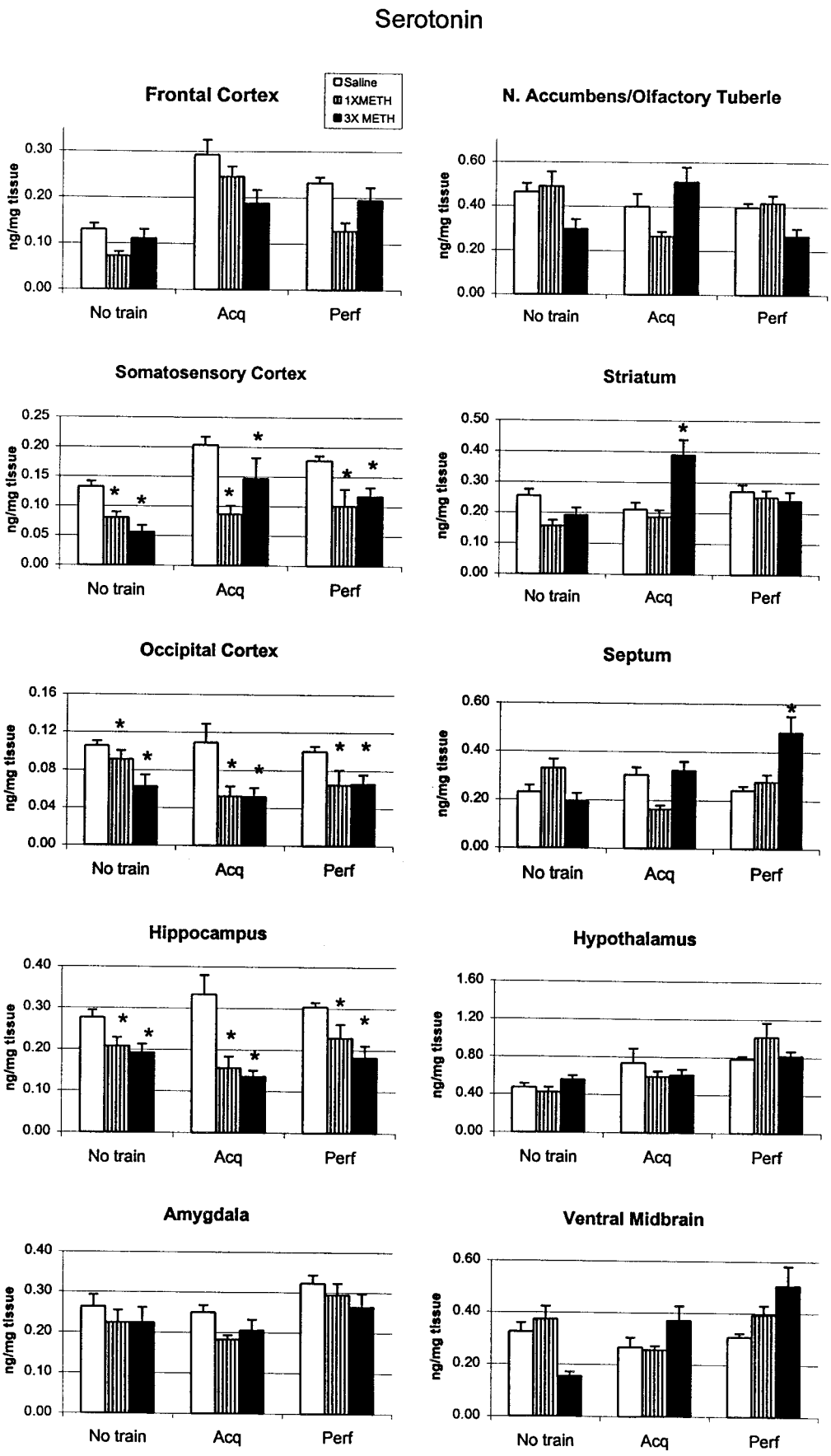

Fig. 4. Effects of high-dose METH pretreatment on postmortem serotonin tissue concentrations. Each regimen consisted of 15 mg/kg/injection, four injections, at 2-h intervals. Animals were sacrificed 33 weeks (3×METH, three-regimen groups) or 47 weeks (1×METH, one-regimen groups) after the last exposure to METH (age 70 weeks). Regimens were separated by 7 weeks. *, significantly different from saline. No train indicates the rats received no DRL training; acq, DRL acquisition at age 60 weeks; perf, DRL performance. Initial training in the performance group occurred before METH treatments began.

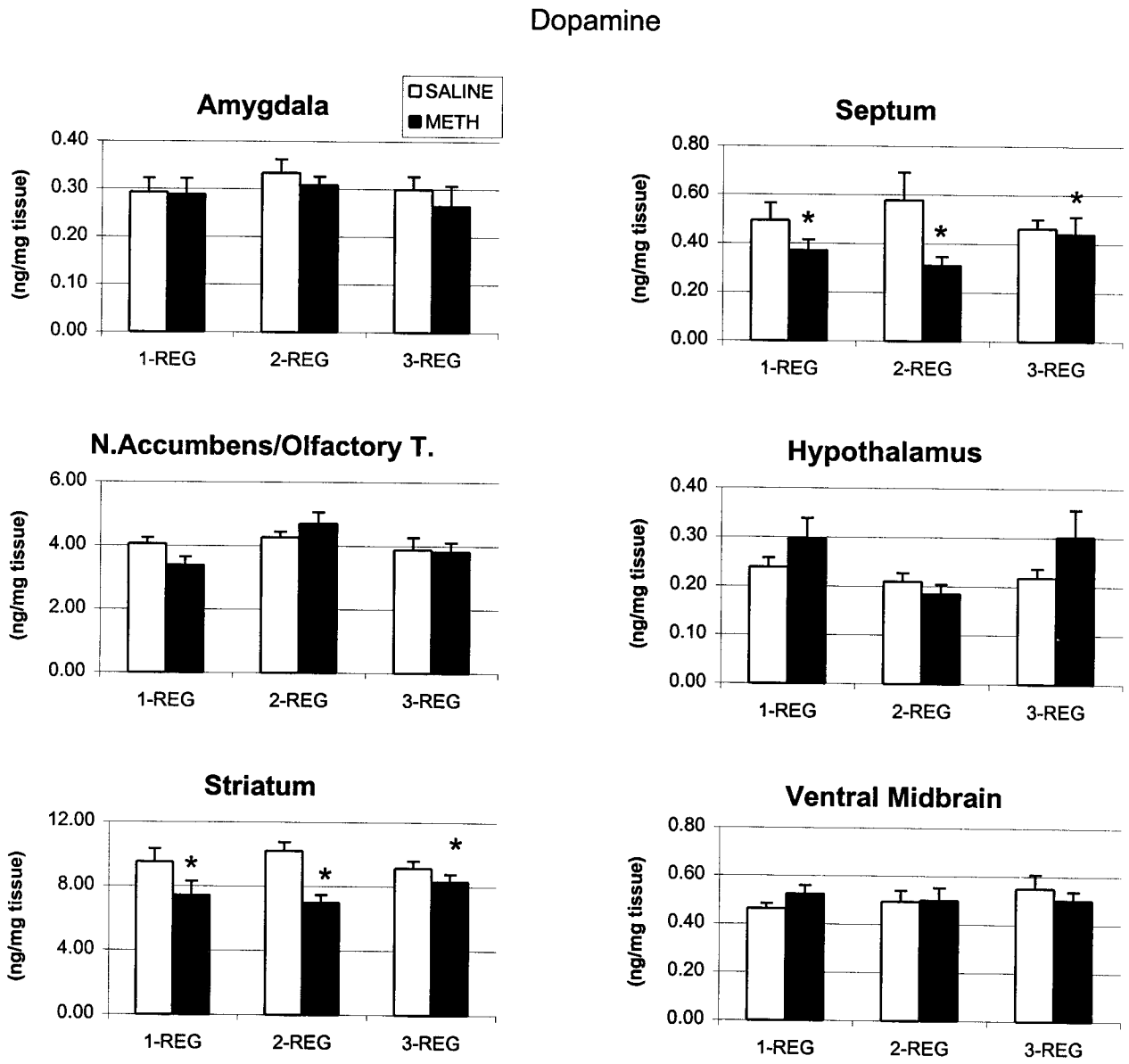

Fig. 5. Dopamine tissue concentrations after one, two, or three regimens of METH (each regimen consisted of 15 mg/kg/injection, four injections, 2-h intervals). Regimens were separated by 7 weeks. Rats were sacrificed at 6 weeks after their last treatment. This corresponded to ages 29, 37, or 43 weeks for the one-, two-, and three-regimen groups, respectively. *, significantly different from saline.

ture of all regimens was used (regardless of regimen number). We found no significant correlations between serotonin and core temperature in frontal cortex, somatosensory cortex, occipital cortex, hippocampus, striatum, or amygdala. We also found no significant correlations between core temperature and dopamine in striatum.

Experiment 5: Neurochemical Measures

Rats were treated with a single high-dose METH regimen and sacrificed 2 weeks after treatment. For dopamine, a significant interaction was identified ($F_{1,9}$ = 22.62, P = .001035). Post hoc analysis indicated that dopamine was decreased in striatum but not nucleus accumbens. For serotonin, significant main effects of treatment ($F_{1,9}$ = 12.72, P = .006) and brain region ($F_{3,27}$ = 16.61, P = .000003) were found; there was no significant interaction. Post hoc analysis on marginal means for treatment confirmed a significant decrease in serotonin (see Fig. 8).

Discussion

Rats were treated with one, two, or three high-dose METH regimens and evaluated on behavioral and neurochemical measures during the aging process.

Behavior. Rats treated with high doses of METH after 12 weeks of DRL 72-s schedule training showed mild impairments on DRL performance. Three weeks after a single METH regimen, response rate was increased and PkA (an index of how well the rats waited for the 72-s interval to elapse) was decreased. Although the effect on PkA was present 14 weeks later, the response rate had returned to normal. Rats treated with 3 METH regimens did not show a

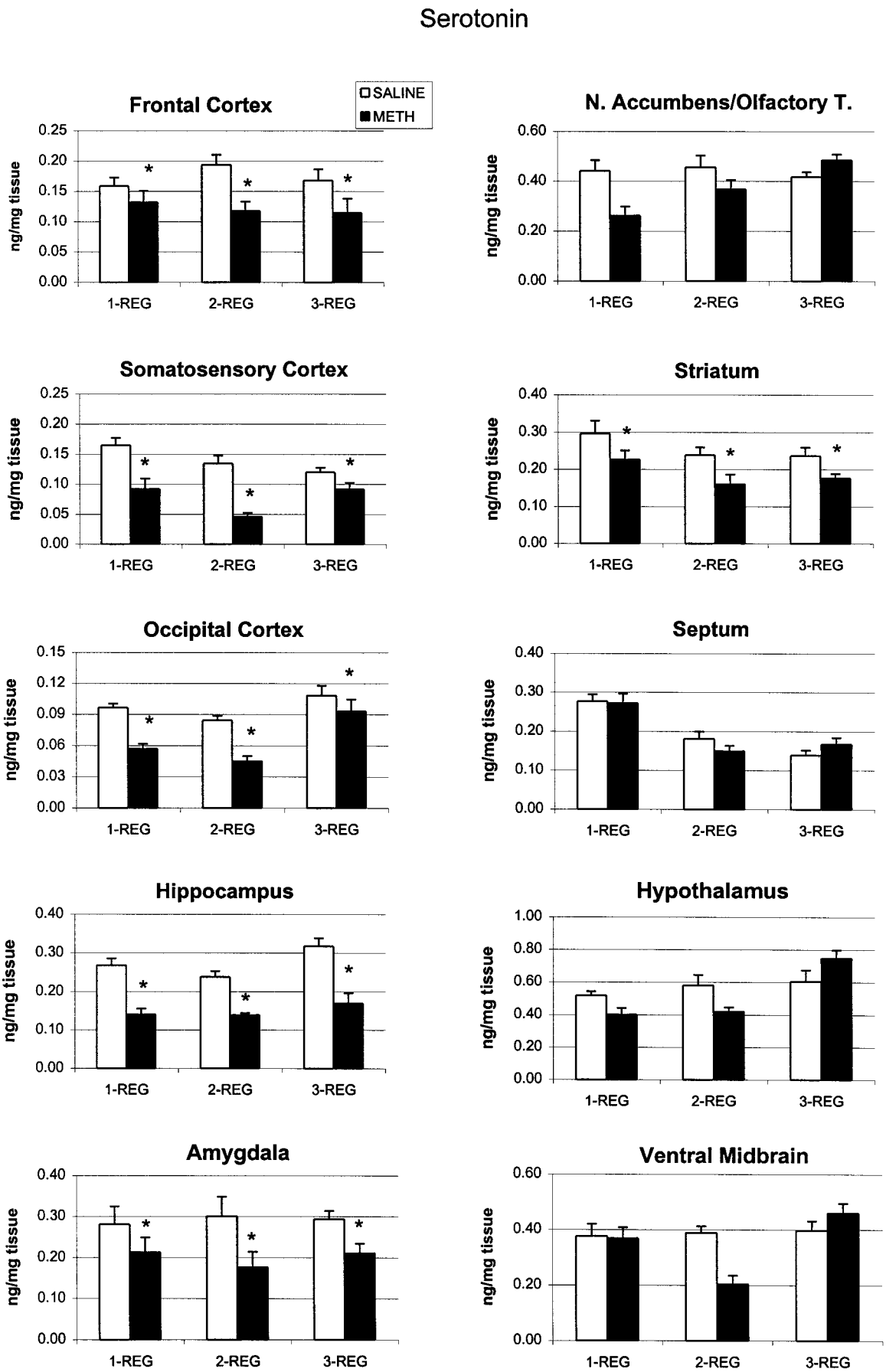

Fig. 6. Serotonin tissue concentrations after one, two, or three regimens of METH (each regimen consisted of 15 mg/kg/injection, four injections, 2-h intervals). Regimens were separated by 7 weeks. Rats were sacrificed at 6 weeks after their last treatment. This corresponded to ages 29, 37, or 43 weeks for the one-, two-, and three-regimen groups, respectively. *, significantly different from saline.

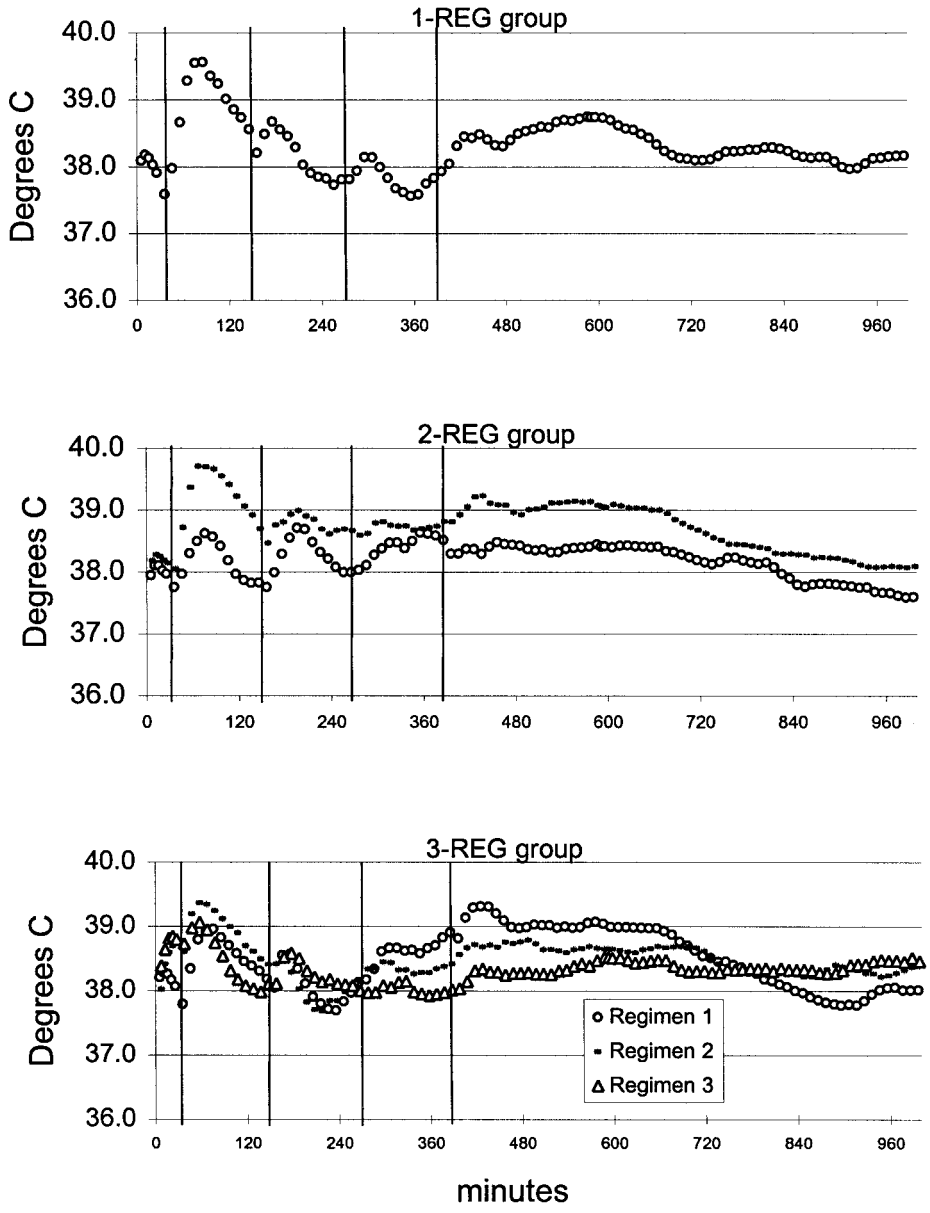

Fig. 7. Core body temperature in response to treatment with one, two, or three METH regimens (each regimen consisted of 15 mg/kg/injection, four injections, 2-h intervals). Regimens were separated by 7 weeks. Vertical lines indicate time of METH administration within regimens.

TABLE 3
Maximum temperature attained
Values are mean ± S.E.

	METH 3-REG GP	METH 2-REG GP	METH 1-REG GP
		°C	
Regimen 1	39.67 ± 0.19	39.68 ± 0.14	39.57 ± 0.16
Regimen 2	39.52 ± 0.10	40.16 ± 0.08	
Regimen 3	39.35 ± 0.08		

GP, group.

greater behavioral impairment than the rats treated with 1 METH regimen (Fig. 1). In neither the METH 1-REG nor the METH 3-REG group did the behavioral impairment extend into the middle-age period of the rat's life (age 62–70 weeks old). When DRL 72-s schedule acquisition was initiated at age 60 weeks, 23 or 37 weeks after METH treatments ended, no behavioral impairments were noted relative to age-matched controls. All groups acquired the task (Fig. 2). The pattern of recovery seen in DRL 72-s schedule performance is consistent with the effects of high-dose METH on the Morris water maze reported by Friedman et al. (1998).

Neurochemistry. No dopamine depletions were noted at 70 weeks of age (Fig. 3, experiments 1, 2, and 3). Serotonin depletions were seen in somatosensory cortex, occipital cortex, and hippocampus (Fig. 4). As was seen in the behavioral findings, the METH 3-REG group did not result in greater neurochemical depletions than the METH 1-REG group. Significant serotonin enhancements were noted in two brain regions: striatum and septum. Significant dopamine enhancement was also noted in the septum. This effect was restricted to METH 3-REG treatment, in animals with either acquisition (striatum) or performance (septum) training. The enhancement was not seen in animals treated with a single regimen or in animals that received no behavioral training (Figs. 3 and 4). The region-dependent increases in serotonin (above controls) after high-dose METH treatment are consistent with previous reports in the literature. Richards et al. (1993a) reported increased hypothalamic serotonin after

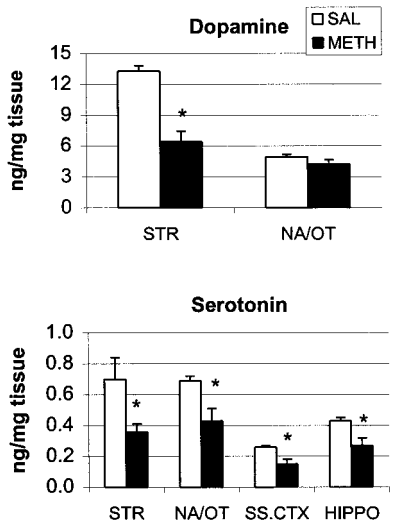

Fig. 8. Dopamine and serotonin tissue concentrations 2 weeks after a high-dose METH regimen (15 mg/kg/injection, four times, 2-h intervals). *, significantly different from saline-treated rats. STR, striatum; NA/OT, nucleus accumbens/olfactory tubercle; SS.CTX, somatosensory cortex; HIPPO, hippocampus.

METH. Ricaurte et al. (1992) and Sabol et al. (1996) reported increased serotonin levels in hypothalamus, septum, or midbrain after 3,4-methylenedioxymethamphetamine treatment. However, single-regimen exposure was used in the previous reports; in this report, we saw only enhanced levels in rats treated with three regimens.

For the dopamine system, there were significant main effects of behavioral training condition on dopamine tissue concentrations, independent of METH treatment. Rats in the no-training condition showed less dopamine than rats in either one or both training conditions in striatum, septum, hypothalamus, and ventral midbrain (Fig. 3). These results suggest that the dopamine system was enhanced by behavioral/environmental experience. Our argument would be strengthened if the acquisition rats (which began behavioral training began in middle age) had intermediate dopamine levels compared with the no-training and performance (training began at age 10 weeks). This was true for septum and hypothalamus but not for striatum and ventral midbrain. The suggestion that dopamine tissue concentrations were affected by experience is made with caution, however, because the rats in the no-training and DRL conditions were raised in different facilities, and the tissue samples were assayed at different times.

In experiment 4, rats were given one, two, or three METH regimens according to the treatment schedule used in the behavioral experiments (experiments 1 and 2). However, each group was analyzed 6 weeks after its last regimen. This 6-week survival period in experiment 4 corresponded with the behavioral test period that occurred after each regimen in experiment 1. Dopamine in the striatum and septum was significantly depleted 6 weeks after one, two, or three METH regimens; the number of regimens did not affect degree of depletion (Fig. 5).

Serotonin was significantly depleted 6 weeks after one, two, or three METH regimens in frontal cortex, somatosensory cortex, occipital cortex, hippocampus, amygdala, and striatum (Fig. 6). In these six brain regions, the degree of METH-induced depletion was not affected by the number of regimens. In the nucleus accumbens/olfactory tubercle and ventral midbrain, there were nonsignificant trends toward differential depletions depending on the number of METH regimens. This trend toward depletion was seen after one or two (but not three) METH regimens, respectively.

Recovery of Neurotransmitters Levels. Striatal dopamine was depleted at 2 weeks post-treatment (experiment 5) and 6 weeks after the last treatment (29, 37, or 43 weeks of age; experiment 4) but not at 33 or 47 weeks after the last treatment (70 weeks of age; experiments 1, 2, and 3). For the serotonin system, depletions at 2 weeks post-treatment were seen in all four regions tested (striatum, nucleus accumbens, somatosensory cortex, and hippocampus). At 6 weeks post-treatment (age 29, 37, or 43), depletions were seen in frontal cortex, somatosensory cortex, occipital cortex, hippocampus, amygdala, and striatum. By 33 or 47 weeks post-treatment (age 70 weeks), only somatosensory cortex, occipital cortex, and hippocampus showed significant depletions. These outcomes indicate recovery over time in both the dopamine and serotonin systems after METH-induced depletions. These findings are consistent with the results of Cass and Manning (1999) and Friedman et al. (1998), who reported recovery of dopamine and serotonin tissue concentrations after long post-treatment intervals.

Relationship between Behavioral Deficit and Neurotransmitter Depletions. Behavioral deficits were seen during the 21 weeks that followed the initial exposure to METH in the performance group (experiment 1). Assay data from experiment 4 indicate that dopamine in striatum and septum and serotonin in frontal cortex, somatosensory cortex, occipital cortex, hippocampus, amygdala, and striatum were depleted at the time of behavioral impairment in experiment 1.

The increase in response rate and the decrease in PkA are similar to the effects reported by Jolly et al. (1999) after 5,7-DHT-induced serotonin depletions. Although the degree of deficit was much greater in the Jolly et al. (1999) report, both the response rate and PkA measures were affected in a similar manner. We therefore interpret our findings to indicate that the METH-induced impairments on DRL 72-s schedule behavior are due to METH-induced serotonin depletions. A role for dopamine depletion in the observed DRL deficit cannot be ruled out, however. Sokolowski and Salamone (1994) reported DRL impairments after 6-hydroxydopamine-induced dopamine depletions of medial prefrontal cortex.

Dissociation between Behavioral Impairments and Neurochemical Depletions. Although there was no sign of behavioral impairment at age 60 to 70 weeks for either the performance group or the acquisition group, significant serotonin depletions were obtained in cortex and hippocampus. The neurochemical recovery discussed earlier may underlie the behavioral recovery, but the degree of serotonin depletion observed in cortex and hippocampus at the end of the experiment was significant. The behavioral recovery may, therefore, have involved other mechanisms (e.g., the animals may have learned to compensate for diminished serotonin levels).

Difference in METH-Induced Depletions across Experiments. In the three experiments in which tissue was analyzed at age 70 weeks, two subcortical structures, striatum and septum, showed neurotransmitter enhancements. Enhanced neurotransmitter concentrations (serotonin in striatum and septum, dopamine in septum) were seen only in

rats that received 3 METH regimens and behavioral training. These findings suggest two possibilities. First, the manner in which the brain recovered from injury was influenced by repeated METH exposure. Second, behavioral experience itself may have enhanced neurotransmitter recovery.

Although the suggestion that 1) multiple regimens and 2) behavioral experience enhanced serotonin/dopamine tissue concentrations is speculative, it is supported by diverse areas of the neuroscience literature. Sprouting of serotonin terminals after treatment with the METH analog *p*-chloroamphetamine has been demonstrated (Mamounas and Molliver, 1991; Mamounas et al., 1992; Wilson et al., 1993). Other experiments, involving the discrete lesion technique, have shown a "priming" effect on subsequent axonal sprouting. When a small priming lesion precedes a larger lesion of entorhinal cortex, axonal sprouting in the deafferented terminal area (hippocampus) is enhanced in comparison to a single large lesion (Scheff et al., 1977; Lynch et al., 1982). In METH-treated rats, the initial regimen may serve a similar priming function. This view is consistent with our findings of METH-induced neurotransmitter enhancement in the 70-week-old rats; in addition, it is consistent with our finding that three METH regimens did not cause greater depletions than 1 regimen. In both cases, the METH 3-REG results may reflect an enhanced recovery process; a compensatory process that may overcome or counteract METH-induced transmitter depletions.

Evidence regarding the role of behavioral experience in neuronal sprouting also comes from diverse fields within neuroscience. For example, the well established effects of enriched environment on neuronal sprouting and behavioral performance demonstrate the role of experience in the nonlesioned animal (Rosenzweig and Bennett, 1996). Working with the dopamine system, Stodgell et al. (1996) found that recovery of striatal dopamine was facilitated by behavioral training after neonatal 6-hydroxydopamine (6-OHDA) lesions. However, behavioral training did not facilitate neurotransmitter recovery when 6-OHDA lesions were administered after weaning (Van Keuren et al., 1998). The results of Van Keuren et al. (1998) argue against a behavioral influence on recovery from the METH-induced neurotransmitter depletions observed in this report. However, the partial depletion after METH treatment may elicit a different behavior/neuronal recovery interaction compared with the larger dopamine depletions seen after 6-OHDA treatment. Although the evidence is mixed, the suggestion can be made that behavioral training on an operant task may enhance neuronal recovery after high-dose METH treatment.

Experiments specifically designed to test the effects of multiple METH exposures, as well as behavioral training, on postmortem neurochemical markers will be needed to further understand the long-term relationship between methamphetamine, behavior, and neurochemistry.

Body Temperature, Cooling, and METH-Induced Neurotransmitter Depletions. Rats were cooled during the METH treatments whenever their body temperature surpassed 39.5°C; otherwise, chamber temperature was maintained at 24°C. It may be argued that body temperature was lowered to the point of protection, causing a smaller depletion than would be predicted to occur without cooling. Bowyer et al. (1994) demonstrated that METH-treated rats that experienced severe hyperthermia (core temperature exceeded 41.3°C) and were temporarily cooled to prevent lethality (15–30 min) showed greater dopamine depletions than rats that did not experience severe hyperthermia. The degree of hyperthermia attained by the animals in the present study was limited by cooling the animals when they reached 39.5°C. The decision to use a lower hyperthermic cutoff in this study was based on a high lethality rate found in pilot animals of similar age. Similarly, the use of 24°C ambient temperature may have limited the extent to which core temperature was increased by METH. Malberg and Seiden (1998) demonstrated a relationship between ambient temperature and serotonin depletions induced by 3,4-methylenedioxymethamphetamine.

Based on data from experiment 4, temperature parameters used in these experiments resulted in average hyperthermic values of 39.4° to 40.2°C. From Table 3, it appears that the larger increase in core temperature in the second regimen of the two-regimen group may have caused greater transmitter depletions. Visual inspection of Fig. 6 suggests that rats in the two-regimen group showed larger depletions of serotonin compared with the one-regimen and three-regimen groups for frontal cortex, somatosensory cortex, occipital cortex, and ventral midbrain. However, statistical analysis did not support this interpretation. The results of correlational analysis indicated there was no relationship between serotonin depletion and core temperature. These findings suggest the following: 1) the parameters for ambient and maximum core temperature used in these experiments held core temperature within a narrow range, minimizing its contribution to the variability in neurotransmitter depletions seen between groups; and 2) the values of these two parameters (24°C ambient and 39.5°C cooling threshold) may have limited the degree of neurotransmitter depletion caused by the dose of METH used in these studies.

Summary. Small behavioral deficits were seen with DRL 72-s schedule performance after one or three high-dose METH regimens. The deficits in performance were not intensified by multiple METH regimens (in comparison with one regimen), nor did they persist into the middle-age period of a rat's life. No deficits were observed when DRL 72-s schedule acquisition was initiated at age 60 weeks, 23 weeks after the last METH treatment.

At the end of the study (at age 70 weeks), serotonin depletions were seen in cortex and hippocampus in the METH 3-REG groups (33 weeks after the third regimen) and METH 1-REG groups (47 weeks after the single regimen). METH 3-REG treatment did not cause greater neurotransmitter depletions than METH 1-REG treatment. The METH 3-REG group, but not the METH 1-REG group, showed enhanced serotonin levels (compared with age-matched controls) in septum and striatum. For the dopamine system, no depletions were observed at age 70 weeks; septal dopamine after METH 3-REG exposure was enhanced. Three high-dose METH regimens may have interacted with behavioral training to cause these enhanced neurotransmitter levels in specific brain regions.

Rats treated with one, two, or three METH regimens showed significant depletions of serotonin in the cortex, hippocampus, amygdala, and striatum when measured 6 weeks after the last regimen. Dopamine in the striatum and septum was depleted after one, two, or three regimens. These findings suggest that the dopamine and serotonin systems of the

parallel DRL groups were decreased at the time of behavioral impairment. For both serotonin and dopamine, increasing the number of METH regimens did not increase the degree of depletion.

Based on previous reports using the selective serotonin neurotoxin 5,7-DHT, it is suggested that the METH-induced serotonin depletions are responsible for the behavioral deficits seen in performance of DRL 72-s schedule after high-dose METH treatment.

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