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Long-Term Monoamine Depletion, Differential Recovery, and Subtle Behavioral Impairment Following Methamphetamine-Induced Neurotoxicity

SETH D. FRIEDMAN,* EDWARD CASTAÑEDA† AND GORDON K. HODGE*

*University of New Mexico, Department of Psychology, Albuquerque, NM, and
†Arizona State University, Department of Psychology, Tempe, AZ

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FRIEDMAN, S. D., E. CASTAÑEDA AND G. K. HODGE. *Long-term monoamine depletion, differential recovery, and subtle behavioral impairment following methamphetamine-induced neurotoxicity.* PHARMACOL BIOCHEM BEHAV **61**(1) 35–44, 1998.—Squads of rats were assayed at three intervals following MA-induced neurotoxicity to investigate the persistence of monoamine deficits, the potential for monoamine recovery, and spatial task abilities. At 48, 139, and 237 days postinjection, MA animals showed significant monoamine depletions compared with controls. Investigating percent depletions (MA/control) across time showed monoamine recovery in some structures. Initially, 5-HT within medial prefrontal cortex (MPFC), caudate (CdN), and hippocampus (HPC) was reduced to 30% of control levels. By 237 days, MPFC and CdN levels were elevated to 70%. Similarly, initial CdN DA reductions (30% of control levels) showed recovery to 80% by 237 days. These findings support neurochemical recovery following MA neurotoxicity. However, the persistent depression of HPC 5-HT suggests that not all structures recover equally. The HPC did show elevated turnover (metabolite/neurotransmitter) over time, suggesting a unique compensatory response. MA treatment also produced an impairment in the Morris water-maze place task at 65 days postinjection. No impairments were observed in water-maze moving platform or place task at 79 and 165 days postinjection, respectively, or in T-maze alternation. The possibility that partial recovery in tissue monamine levels underlies the sparing of function and behavioral improvement is discussed. © 1998 Elsevier Science Inc.

Long-term monoamine depletion Behavioral impairment Methamphetamine-induced neurotoxicity

AT high doses, peripheral injections of methamphetamine (MA) are selectively neurotoxic to dopaminergic and serotonergic systems in rat brain (8,12,13,26,31). Damage from MA-induced neurotoxicity has been shown to be dose dependent (38), and a function of the duration MA remains in the system (31).

With MA doses sufficient to damage both dopamine (DA) and serotonin (5-HT) systems, characteristic physiological effects are found. Substantial nerve terminal degeneration occurs within DA neurons (18,21,22,25) and 5-HT neurons (24,31), although the destruction of cell bodies remains unclear (23,34). Substantial depletions in the major production enzymes of DA and 5-HT, tyrosine hydroxylase (5,12,15) and tryptophan hydroxylase (12), are found following repeated MA administration. Reductions in the extracellular concentrations of the DA metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA) have been demonstrated following MA neurotoxicity (29,30) as well as postmortem 5-HIAA levels (1).

MA neurotoxicity in rats has been shown to produce long-term depletions (up to 180 days) of both DA (2,26) and 5-HT (24,26). In primate investigations, persistent depletions in DA and 5-HT have been shown up to 4 years following high-dose

Requests for reprints should be addressed to Dr. Seth D. Friedman, Department of Outpatient Psychiatry, University of Washington School of Medicine, 4225 Roosevelt Way NE, Suite 306c, Seattle, WA 98105-6099.

MA treatment (42). Seiden (32) suggests that partial monoamine depletions following MA-induced neurotoxicity may be compensated for while animals are young, but become an increasing problem as animals age. However, the extent of recovery of monoamine depletion following MA treatment has not been investigated, nor is it known whether rats show monoamine deficits for longer than 180 days after treatment. In the current study, we investigated these questions by studying three squads of animals following MA-induced neurotoxicity to characterize depleted states of tissue monoamine levels at varying intervals following drug treatment, and to assess potential recovery in these monoamine levels from neurotoxic MA exposure.

A further consequence of neurotoxic doses of MA is behavioral impairment. However, deficits have not been shown in the majority of tasks. In a comprehensive test battery investigating feeding and drinking, open-field behavior, one-way and two-way avoidance, and forced swimming, no impairments were found on any task following MA treatment (32). Behavioral impairments in active-avoidance (39), balance beam (39), and reaction time tasks (26) have been reported following MA-induced neurotoxicity. In our laboratory, we have shown deficits on T-maze alternation and touch-screen alternation using albino animals (4,7). The present study also sought to investigate whether Long–Evans rats, a strain having superior vision to albino animals (9) used previously, showed deficits on spatial tasks following MA-induced neurotoxicity.

METHOD

Animals

Forty-eight male Long–Evans (Harlan Sprague–Dawley, South Carolina) rats weighing between 283–362 g on day 60 postpartum were used. Rats were housed individually in hanging metal cages at room temperature (21°C) under a 12 L:12 D cycle (lights off at 1900 h). Behavioral testing was conducted during the latter half of the light cycle. Food and water were provided ad lib unless otherwise noted below. To make this study feasible animals were run in squads. The protocol for this study was approved by the University of New Mexico Animal Care and Use Committee. Experimenters were kept blind to group designation throughout all testing.

Drug Treatment

At 60 days of age, animals were randomly assigned to MA treatment ($n = 25$) or control ($n = 23$) groups. A neurotoxic schedule of methamphetamine•HCl (12.5 mg/kg; NIDA-RTB, Rockville, MD) was administered, consisting of four SC injections with 2-h intervals between injections (39). Control animals underwent an identical injection regimen of vehicle (0.9% saline, 1 ml/kg). Because temperature is an important contributor to MA-induced neurotoxicity (3), room temperature was held constant (24°C) during the treatment regimen and drug washout for all animals studied.

Design

Three groups of animals were assayed approximately 100 days apart to investigate monoamine depletions over time and spatial task performance. Squad A (six MA, six control) was assayed at 48 days post-MA treatment to assess the severity of monoamine depletions following MA-induced neurotoxicity. Squad B (seven MA, five control) was assayed at 139 days post-MA treatment following Morris water-maze place and moving platform tests. Squad C (14 MA, 10 controls) was assayed at 237 days post-MA treatment following T-maze and Morris water-maze place tests. One animal from Squad C died as a result of the MA treatment.

Tissue Assay

A postmortem tissue analysis for brain monoamine levels was conducted as described by Robinson et al. (28). Rats were killed by decapitation and their brains removed within 45 s and placed in ice-cold physiological saline. After allowing each brain to cool for 30–45 s it was placed on a chilled cutting block. Brain slices were obtained by the method of Heffner (11). Medial prefrontal cortex (MPFC) was transected out, the caudate nucleus (CdN) was removed by using a 2-mm tissue punch, and the hippocampus (HPC) freely dissected. Each brain region was dissected bilaterally and both pieces weighed together. MPFC, CdN, and HPC samples were placed into microcentrifuge tubes containing 200, 400, and 800 μl, respectively, of 0.05 N $HClO_4$ and dihydroxybenzylamine (1 ng/μl). The samples were homogenized and centrifuged (4000 rpm at 4°C for 25 min) and the supernatant was filtered (0.2 μm) before storing at −20°C.

The supernatant was injected into a 10 cm length Rainin Microsorb-MV (3 mm, C18 particles, 4.6 mm i.d.) column (Rainin, Emeryville, CA). The mobile phase consisted of 60 mM NaH_2PO_4, 30 mM citric acid, 0.1 mM EDTA, 32 mg/l sodium dodecyl sulfate, and 32% MeOH, at a pH of 3.8. A dual electrode Model 5011 High Sensitivity Analytical Cell and Model 5200 Coulochem II detector (ESA, Chelmsford, MA) was used to collect chromatographic data. A conditioning cell (ESA Model 5021) was set to oxidize at +100 mV. The oxidation of DA, DOPAC, HVA, norepinephrine (NE), 5-HT, and 5-HIAA was measured by the first analytical cell set to +340 mV and 200 nA sensitivity. Following oxidation at the first electrode, reduction at the second electrode, set to −350 mV and 5 nA sensitivity, was used to measure DA and 5-HT when these were not measurable on the oxidation signal. Peak heights were recorded on a Linear Model 1200 dual pen recorder (Alltech, Deerfield, IL). Peaks from six serially diluted standards were used to calculate the concentration of the monoamines based on linear regression analyses.

PROCEDURES

Morris Water-Maze

Squad B (seven MA, five control) and Squad C (13 MA, 10 control) were tested in identical Morris water-maze place tests at 65 days postinjection and 165 days postinjection, respectively.

Apparatus

The maze consisted of a circular pool (1.5 m diameter by 45 cm in height); the inner pool surface was painted white. The pool was filled daily with water to a depth of 25 cm, which was made opaque by adding instant skim milk. Water temperature for all water-maze tasks was approximately room temperature (24°C). The hidden platform was constructed of Plexiglas (11 × 12 cm) and submerged 1 cm below the water surface. A computer system [see (16)] was used to track the rat during swimming trials and to collect angle information (the rat's angle relative to the platform 12 cm after release), swim path, and distance information. Escape latency was recorded by the experimenter.

Place Task

Animals were given two blocks of four trials daily for 5 days, with each block of trials including a North, South, East, and West starting position in random order. The hidden platform was located in the center of the NW quadrant for the first five trial blocks (2-1/2 days) and then was moved to the NE quadrant of the pool for the remaining five blocks of trials. One probe trial, which consisted of 20 s swimming without a platform to assess pool quadrant preference, was conducted at the end of trial blocks 5 and 10.

At the beginning of each trial, animals were gently placed in the water facing toward the pool wall. Timing began when the rat was released. If the rat found the platform within 60 s, the rat was allowed 10 s to stand on the platform, and was then removed from the pool and placed into its home cage, which was covered with an opaque lid on an animal cart. If the rat failed to find the platform within 60 s, the animal was removed from the pool without platform exposure. Rats were run in sets of six. After a trial, each rat was administered a subsequent trial only after all other rats in that set were tested in the same trial.

Moving Platform Task

Squad B (seven MA, five control) was tested in the moving platform task at 79 days postinjection.

In this phase of the experiment, animals were tested in a room different from the one in which place testing occurred. Subjects were given two blocks of trials each day similar to the place task except that platform location changed on the first trial of each day and no platform location was identical between test days. Rats were tested for 10 days in this room, but then on 2 additional days testing was conducted in another novel room (35,40). Heading angle and distance data were collected manually on these latter 2 days.

T-Maze Task

Squad C, consisting of 23 animals (13 MA, 10 controls), was food restricted beginning 2 weeks post-MA treatment to 85–90% of normal body weight. Upon completion of T-maze testing animals were returned to an ad lib feed schedule.

Apparatus

The T-maze was constructed of clear 0.5 cm Plexiglas with all arms measuring 12 cm in height and 11 cm in width. The lengths for the start box, stem, and arms were 26, 58, and 36 cm, respectively, and each was covered with hinged lids. To restrict rats within specific areas of the T-maze, a clear guillotine door separated the start box from the rest of the maze and others were located at the entrance to each arm. Located at the end of each goal arm was an opaque guillotine door that could be raised by the experimenter to provide food reward in the form of a Cheerio (General Mills Foods). Both food magazines were baited throughout testing to ensure equivalent odor cues.

Behavioral Testing

Fourteen days post-MA treatment, animals were habituated to the T-maze over 4 days. On days 1 and 2, animals were given free access to the maze for 10 min with 10 Cheerios scattered across goal arms. On day 3, animals were each given a total of 10 trials (five left, five right) with one goal arm blocked and a Cheerio placed before the food magazine near the end of the accessible goal arm. On day 4, the 10 forced choice trials were repeated except the Cheerio was hidden behind the food magazine. The criterion for receiving reward on this day, and subsequently, was entry into the correct alley with all four feet.

On day 5, alternation testing began. Each trial consisted of a forced choice and a subsequent two-choice task. Animals were given six pseudorandom (three left, three right, in random order with no more than two in a row in the same direction) trials per day for days 1–7.

The forced trial started by raising the start box door and ended upon entry into the open arm, which resulted in reinforcement and restriction in the forced arm for 20 s. The rat was immediately returned to the start box for the choice trial, in which access to both goal arms was allowed. A correct response was defined as entry into the opposite arm from the previous forced choice trial and was reinforced. Incorrect responses were not reinforced. Regardless of the two-choice response, animals were restricted to the chosen arm for 20 s. Before the next trial, animals were removed from the maze and placed into an opaque box ($20 \times 25 \times 20$ cm) for a 1-min intertrial interval (ITI). Animals were run to an average 94% criterion across 3 consecutive days.

Following alternation testing the task was made more difficult in three ways. First, a 1-min delay between the forced and choice trials in which the animal waited in the start box was introduced. This necessitated testing the animals over 2 days. Each animal received six trials of this manipulation. Second, across the next six trials for each animal, odor cues were also removed by wiping the goal arms with a damp sponge. Over the next 21 days of testing, a third manipulation was carried out to minimize distal cues. Instead of waiting in the start box during the 1-min delay, animals were removed from the maze and placed into their home cage covered with an opaque lid. The entire maze was also cleaned out for odor cues during this delay.

Finally, rats were returned to a daily running schedule for testing in a match-to-position paradigm. Rats were required to choose the same arm visited on the forced trial to receive reward. During this task the delay between force and choice trials was removed.

RESULTS

Neurochemical Measures

Table 1 shows postmortem monoamine tissue levels and pairwise comparisons (planned t-tests) between controls and MA-treated rats conducted at the three intervals examined following drug treatment. All animals in Squad A (48 days) and Squad B (139 days), but only 12 randomly selected (six control, six MA) rats from Squad C (237 days), were used in this analysis. As indicated, there were significant depletions in MA-treated groups compared to controls. Both 5-HT and 5-HIAA were depleted across all structures at 48 and 139 days. These depletions were still observed at 237 days in the CdN and HPC; however, not in the MPFC. For DA and DOPAC no significant effects of MA treatment were found, except for reductions in the CdN at 48 days and 237 days. Trace amounts of DA and DOPAC were detectable in the HPC, but variation from signal-to-noise did not allow a reliable measure to detect a difference between groups. Further, because there is thought to be minimal DA within HPC these data are not reported. One MA-treated animal from the 237-day group was removed from analyses of these latter two compounds because values were 10 SD above the mean value. Decreases in

TABLE 1

SUMMARY OF MEAN POST-MORTEM MONAMINE TISSUE LEVELS (ng/mg WET BRAIN TISSUE), STANDARD ERROR, T STATISTIC, AND STATISTICAL SIGNIFICANCE ACROSS STRUCTURES ASSAYED AT 48, 139, AND 237 D POST-MA TREATMENT

	Control	MA	T	Signif
48d				
MPFC				
5HT	1.02 ± 0.14	0.32 ± 0.15	3.40	†
5HIAA	0.16 ± 0.05	0.05 ± 0.02	4.91	‡
DA	0.15 ± 0.007	0.13 ± 0.03	0.41	
DOPAC	NM	NM	NM	
HVA	0.04 ± 0.005	0.03 ± 0.004	2.60	*
CdN				
5HT	0.43 ± 0.09	0.12 ± 0.05	3.18	†
5HIAA	0.38 ± 0.04	0.13 ± 0.05	3.61	†
DA	14.50 ± 1.49	6.00 ± 0.95	4.81	‡
DOPAC	2.71 ± 0.35	1.27 ± 0.22	3.48	†
HVA	1.05 ± 0.19	0.48 ± 0.05	2.97	*
HPC				
5HT	0.62 ± 0.07	0.15 ± 0.07	4.67	‡
5HIAA	0.21 ± 0.02	0.05 ± 0.02	5.13	‡
139d				
MPFC				
5HT	0.29 ± 0.12	0.062 ± 0.02	2.27	*
5HIAA	0.16 ± 0.05	0.052 ± 0.02	2.18	*
DA	0.13 ± 0.01	0.16 ± 0.02	−1.48	
DOPAC	NM	NM	NM	
HVA	NM	NM	NM	
CdN				
5HT	0.12 ± 0.03	0.061 ± 0.01	2.33	*
5HIAA	0.20 ± 0.05	0.10 ± 0.01	2.51	*
DA	12.40 ± 1.76	10.00 ± 1.41	1.08	
DOPAC	1.96 ± 0.10	1.63 ± 0.30	0.88	
HVA	NM	NM	NM	
HPC				
5HT	0.85 ± 0.25	0.27 ± 0.06	2.72	*
5HIAA	0.58 ± 0.07	0.15 ± 0.04	6.22	‡
237d				
MPFC				
5HT	0.20 ± 0.04	0.15 ± 0.03	1.09	
5HIAA	0.10 ± 0.01	0.08 ± 0.01	0.96	
DA	0.14 ± 0.01	0.11 ± 0.008	1.63	
DOPAC	NM	NM	NM	
HVA	NM	NM	NM	
CdN				
5HT	0.12 ± 0.008	0.08 ± 0.01	2.56	*
5HIAA	0.18 ± 0.02	0.11 ± 0.01	2.91	*
DA	10.70 ± 0.40	8.70 ± 0.54	2.99	*
DOPAC	1.98 ± 0.11	1.62 ± 0.07	2.80	*
HVA	NM	NM	NM	
HPC				
5HT	1.28 ± 0.26	0.27 ± 0.06	3.75	†
5HIAA	0.51 ± 0.06	0.14 ± 0.03	5.51	†

*$p < 0.001$, †$p < 0.01$, ‡$p < 0.05$.

HVA were measured in the MPFC and CdN at 48 days postinjection, but unfortunately, were not measurable on later assays. Finally, norepinephrine measured only on the 48-day group showed no significant depletions in the MPFC or HPC (data not shown).

A second set of analyses assessed monoamine turnover across recovery using 5-HIAA/5-HT, DOPAC/DA, and HVA/DA ratios. HPC turnover of 5-HT was significantly reduced at 48 days, $t(10) = 2.75$, $p < 0.05$, not significantly different from controls at 139 days, $t(10) = 0.22$, $p > 0.05$, and elevated at 237 days post-MA treatment, $t(10) = 2.38$, $p < 0.05$. However, no significant differences were found at any recovery interval in the MPFC or CdN. No significant changes in DA turnover (DOPAC/DA and/or HVA/DA) were found in any structure at any time. DA turnover was not assessed at 48 days in HPC, nor at 139 or 237 days in MPFC because DA metabolites were nondetectable.

Comparison of Neurochemical Measures Across Recovery

To investigate changes in neurochemical content over time the data were normalized. We were concerned that performing assays at different time points could produce variable results, for example, due to interassay variability, time of year, etc. Indeed, control rats killed at day 48 showed higher MPFC 5-HT levels than control rats killed at later time points, $F(2, 14) = 16.82$, $p < 0.01$. To control for these effects, the neurochemistry for MA-treated animals was expressed as a percent of controls assayed at each recovery interval.

One-way ANOVAs conducted across recovery times using percent of control data showed there were significant increases towards control levels for 5-HT within MPFC and CdN and for 5-HIAA within MPFC, $F(2, 16) = 5.80$, $p < 0.01$, $F(2,16) = 4.12$, $p < 0.04$, and $F(2, 16) = 5.37$, $p < 0.02$, respectively (see Fig. 1).

At 48 days all regions showed approximately 70% depletions of both 5-HT and 5-HIAA. There was no significant change in these levels at 139 days. By 237 days, 5-HT was significantly enhanced compared with 48 day levels in MPFC and CdN (Newman–Keuls, ps < 0.05), as well as 5-HIAA in MPFC only (Newman–Keuls, $p < 0.05$). By 237 days 5-HT levels had returned to approximately 70% of control values within these two structures, but 5-HT levels in the HPC remained approximately 30% of control values. No statistically significant enhancement in 5-HIAA was measured in the CdN or HPC.

One-way ANOVAs comparing catecholamine levels over time showed significant increases within only CdN DA, $F(2, 15) = 6.24$, $p < 0.01$, and CdN DOPAC, $F(2, 16) = 3.39$, $p = 0.06$ (see Fig. 2. At 48 days CdN DA and DOPAC levels were approximately 40% of control levels. By 139 days, a significant increase of DA was evidenced (Newman–Keuls, $p < 0.05$); however, DOPAC levels were not statistically elevated. By 237 days, both CdN DA and DOPAC had increased to approximately 80% of controls, a significant enhancement from 48 days (Newman–Keuls, ps < 0.05).

Finally, time-course analyses of turnover indices demonstrated that HPC 5-HT turnover was significantly elevated across recovery intervals, $F(2, 16) = 14.13$, $p < 0.01$. Post hoc tests revealed significant increases at all intervals, 48 to 139 days, 48 to 237 days, and 139 to 237 days (Newman–Keuls, $p < 0.05$). No other significant changes in 5-HT or DA turnover were found in other structures.

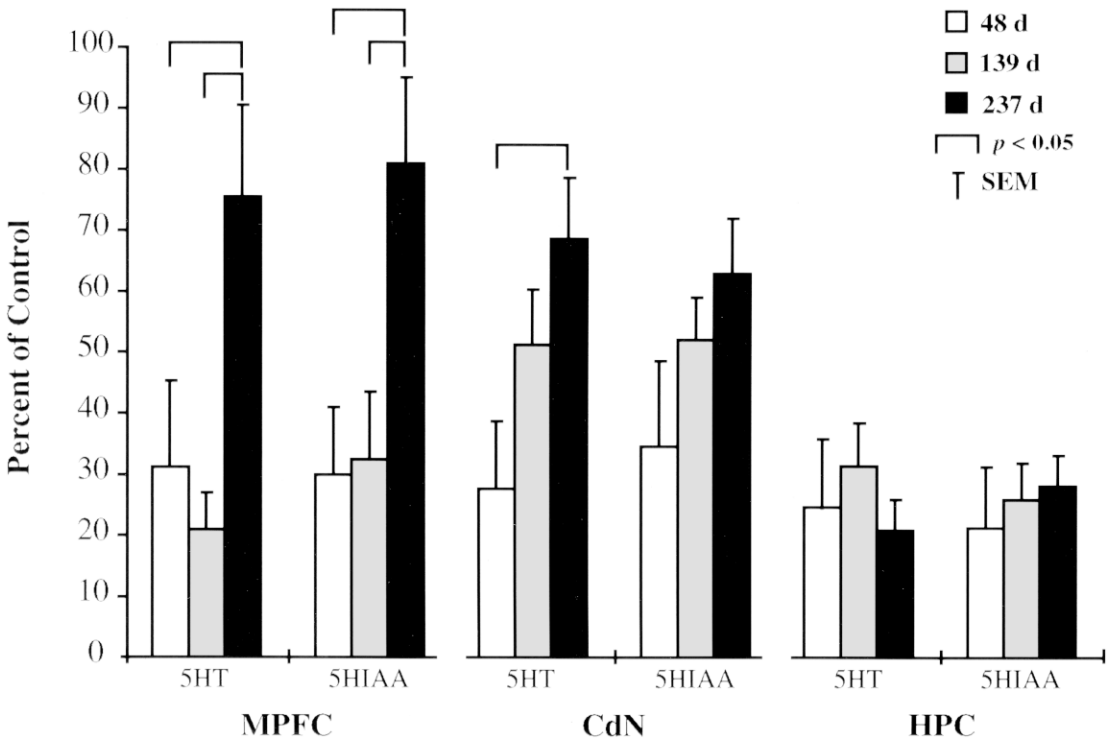

FIG. 1. Recovery of 5-HT and 5-HIAA over time following MA-induced neurotoxicity. Significant enhancement relative to control levels is shown within the MPFC and CdN over time (48–237 days). In contrast, HPC 5-HT and 5-HIAA levels remain persistently depleted over time.

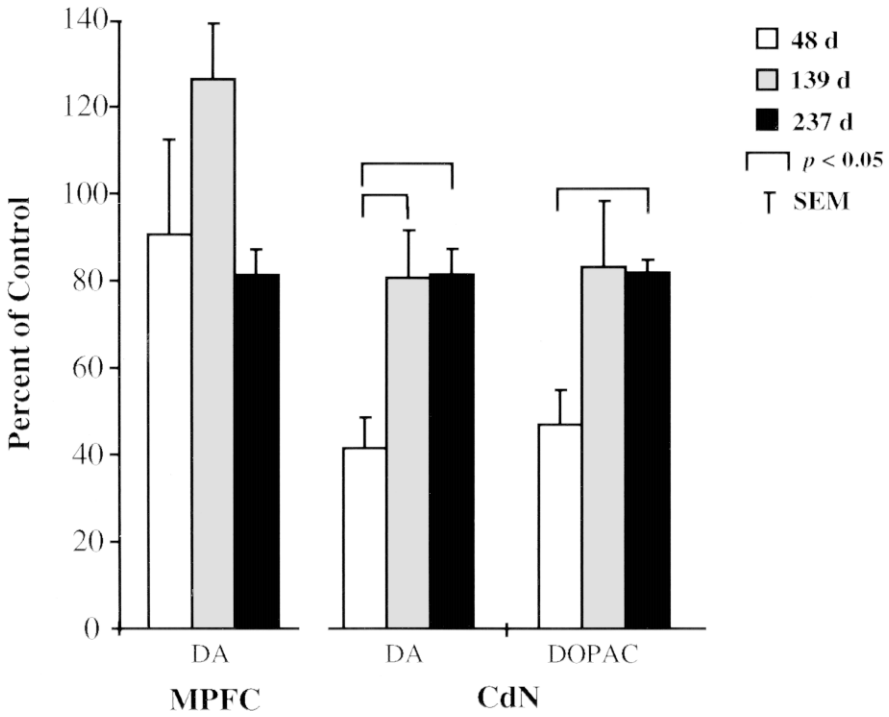

FIG. 2. Recovery of DA and DOPAC levels over time following MA treatment relative to control values. Significant enhancement in CdN DA is shown over the first time interval. Further, significant increases are shown in both CdN DA and DOPAC at 237 days relative to initial depletions found at 48 days.

Place Task—Squad B (65 Days PostInjection)

The latency to escape onto the hidden platform was analyzed by repeated-measures (RM) ANOVA. A group × block interaction was found in the place task, $F(9, 90) = 2.11$, $p < 0.04$, and Newman–Keuls follow-up tests revealed a significant difference between groups on block 2, $q(4) = 4.29$, $p < 0.05$, and a trend approximating a group difference on block 3, $q(4) = 3.25$, $p < 0.10$ (see Fig. 3). MA-treated animals demonstrated markedly impaired escape latency compared with controls on this second block. Further, across groups a significant effect for block, $F(9, 90) = 41.71$, $p < 0.01$, was shown; however, no main group effect was found.

Heading angle and Probe 1 data (no platform present during swimming trial) collected after the first five blocks were not interpretable because of a computer malfunction. Analysis of heading angle over the last five blocks revealed no significant differences between groups or group by block. A significant effect of block, $F(4, 40) = 34.56$, $p < 0.01$, showed that heading angle diminished across the last five blocks in both groups. No significant differences between groups was demonstrated in swim distance across the last five blocks. Analyses by *t*-tests of probe 2 data collected after block 10 revealed no significant group differences across total swim distance, heading angle, or swimming distance within the quadrant previously containing the platform.

Moving Platform Task at 79 Days PostInjection

RM ANOVA analyses were conducted. For escape latency, a significant effect of day, $F(9, 162) = 40.89$, $p < 0.01$, was found; however, no significant differences between groups or group × day was shown across the first 10 test days. Escape latency reached asymptote by day 6. To investigate whether groups solved the task similarly within a day, all trials within a single day were averaged for days 6–10 (i.e., asymptotic latency performance). No significant differences between groups in acquisition rate across this composite block were found.

A number of analyses were conducted to assess ability to transfer an acquired search strategy to a novel context, ability to encode environmental cues, and sensitivity to proactive interference. First, a comparison between escape latencies on the last swimming trial (trial 8) of day 10 and trial 1 of day 11, the first day of testing in a novel environment, should reflect whether a change in test context impairs search strategy. No significant differences were seen between groups or group × day. Second, escape latencies on trial 1 and trial 2 of day 11 were compared to assess ability to encode novel room cues. No significant difference between groups was shown. A third analysis compared the MA-treated group to controls on escape latencies of trial 1 on day 12 to investigate if MA treated animals displayed greater proactive interference from the previous day's correct platform position (i.e., perseveration). No group difference was found. Finally, no difference between groups in heading angle or swim distance was demonstrated in the new test room on any trial of days 11 or 12.

Place Task Performance at 165 Days Postinjection

Eleven MA and eight controls were run in the place task. Two animals from each group were not included because they failed to complete T-maze testing. This criterion was imposed to ensure equivalent spatial cue experience of animals at the

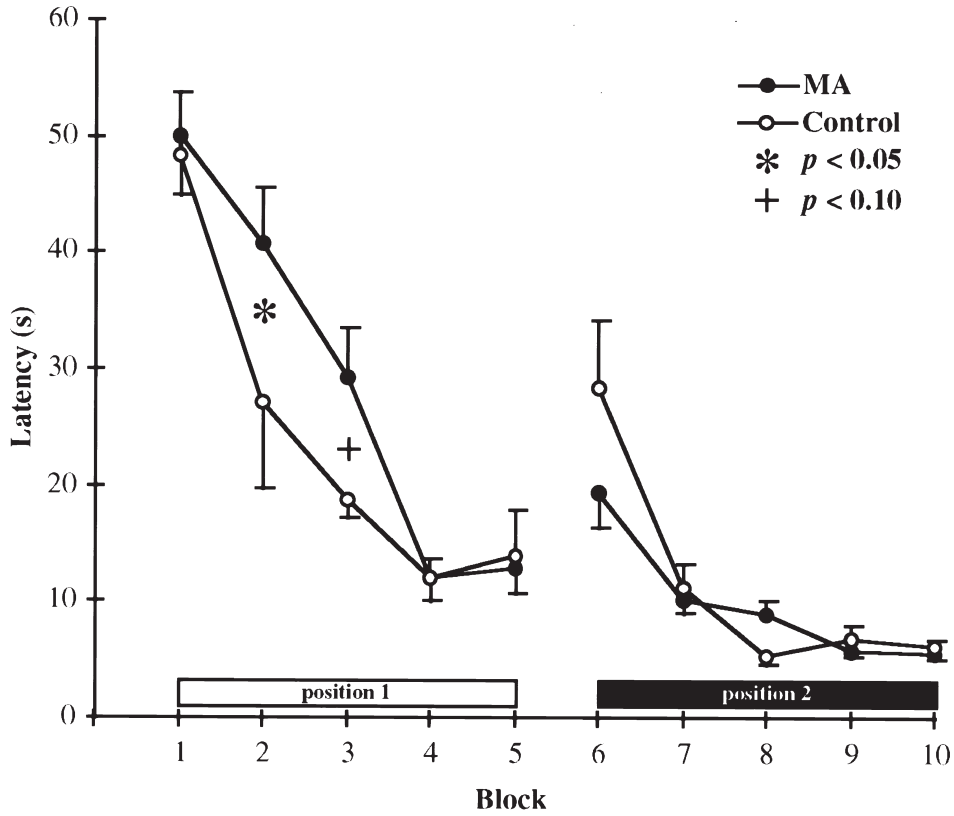

FIG. 3. Escape latency to find the hidden platform in the Morris water-maze place task at 65 days post MA treatment. MA-treated animals were impaired at learning the first platform location because they were significantly slower than controls on block 2 of testing.

start of water-maze testing. RM ANOVA analyses were performed. Although a significant effect of block, $F(9, 162) = 40.89$, $p < 0.01$, was displayed, no significant differences between group or group × day were shown. Analysis of swim distance between groups did not show significant differences.

A significant effect of block on heading angle was found, $F(7, 126) = 15.13$, $p < 0.01$, but no differences between groups or group by block were demonstrated.

Probe data were analyzed by *t*-tests. No significant differences in probe data were shown after trial blocks 5 and 10 for total swim distance, heading angle, or swimming distance within the quadrant previously containing the platform.

T-Maze Performance at 14–100 Days Postinjection

T-maze data were analyzed by RM ANOVA. No significant difference between control and MA-treated animals was found during alternation testing across days 1–7 (to 94% criterion). The introduction of a 1-min delay on day 8/9 did not impair performance of either group from day 7 levels. In addition to the 1-min delay, controlling for odor cues on day 10/11 also did not impair group performance. On day 12/13 animals were additionally removed from the maze and placed in a distractor cage during the 1-min delay. This manipulation impaired animal performance to approximately naive levels (i.e., 75%), as a significant effect of day was found, $F(1,41) = 8.05$, $p < 0.01$. To see whether this increased demand on memory would produce a reacquisition impairment in MA treated animals, 21 further days of testing were imposed. Performance by the end of testing did not improve in either group.

During the match-to-position phase, group performance did improve over the 3 weeks of testing, $F(20, 351) = 3.51$, $p < 0.01$, and both groups reached only 25% correct by the end of testing. No group differences were seen across testing.

DISCUSSION

General Findings

In the present study, MA-treated animals displayed significant monamine depletions compared with controls at 48, 139, and even 237 days postinjection. Interestingly, comparing percent depletions (MA/control) across time points showed substantial recovery in some structures. For example, 5-HT was reduced to 30% of control levels across all structures at 48 days. By 237 days, both the MPFC and CdN evidenced significant enhancement towards control levels (to approximately 70%), but not the HPC. Similarly, 5-HIAA within the MPFC and CdN had increased as well. Moreover, DA depletion of approximately 60% was only observed in the CdN at 48 days, and showed significant increase to approximately 80% by 139 days. This was also accompanied by a similar rate of increase in CdN DOPAC levels. The finding that CdN DA, as well as DOPAC levels, is not significantly reduced on day 139 but decreased on day 237, is apparently due to the increased variance on day 139, because the percent reduction in DA at these two time points is almost identical. It is reasonable to

suggest from these data that there was recovery from about 60% depletion at 48 days to about 20% at 139 days, but that recovery plateaued at this level because no further increase was observed on day 237. The present data support the idea that, despite partial recovery, there is permanent dopaminergic terminal loss in the CdN. Additionally, the finding that HPC 5-HT levels do not increase at either 139 or 237 days suggests that not all structures recover equally.

Neurotoxic doses of MA also produced an impairment in the Morris water-maze place task at 65 days postinjection. However, no impairments were observed in the moving platform or place task at 79 days and 165 days postinjection, respectively. The T-maze protocol did not reveal any deficits. The changes in monoamine levels reported in the present experiment may represent a biochemical compensation that underlies both the large sparing of function, as well as the observed behavioral improvement.

Morris Place Task (65 and 165 Days Postinjection)

MA-treated animals displayed an acquisition impairment in the Morris water-maze place task 65 days postinjection and were specifically slower than controls locating the platform on block 2 and at the trend level on block 3. No differences were seen at other blocks or probe 2, suggesting that MA-treated animals were generally able to utilize room cues following training on the task. This is in contrast to the results seen by Vorhees (37) in which MA-exposed neonates were impaired at initial platform acquisition and reacquisition once the platform was shifted. Differences in rat strain, developmental state, and MA dose could explain the inconsistency between the present study and the latter. Interestingly, in the present study when animals were tested in the place task at 165 days postinjection no impairments were found, suggestive of recovery.

Moving Platform Task (79 Days Postinjection)

To increase task demand, a moving platform task (35,40) was used to test Squad B at 79 days postinjection. The moving platform task shares most of the fixed platform task characteristics with the exception that the hidden platform is placed in a new pool location at the start of each day. This manipulation assesses the rat's ability to utilize familiar contextual information each day to locate the hidden platform. Following overtraining, animals were tested for their ability to encode new spatial information and to transfer a search strategy to a novel context. Because MA-treated animals demonstrated no deficits compared to controls, this suggests that spatial encoding and its use were not impaired at 79 days postinjection.

T-Maze Testing

The significant impairment in acquisition found in T-maze performance (4) was not replicated with the Long–Evans strain. The performance deficit may have been masked by the higher naive alternation performance demonstrated by Long–Evans strain rats (75% correct), and/or a contribution of enhanced visual acuity with respect to navigating to spatial cues. Additional manipulations to make this task more difficult did not reveal any deficits.

Summary of Behavioral Results

The water-maze place task at 65 days postinjection revealed an acquisition deficit in MA-treated animals. Over the same time course no deficit was shown in T-maze testing. The unique contribution of water-maze testing may have been the increased difficulty of the task, i.e., requiring the animal to navigate to a hidden platform from differing starting locations compared with a static starting location relative to room cues in the T-maze, and the increased amount of spatial information required to accurately navigate towards the goal in the water-maze.

Recovery Within the HPC

Although 5-HT in the MPFC and CdN increased from 30 to 70% by 237 days, there was no elevation measured in HPC. The concentration of neurotransmitter is thought to be linearly related to the number of terminals (6,43), so the increases in 5-HT within MPFC and CdN suggest an increase in terminals. Indeed, sprouting of 5-HT terminals has been observed following different types of neurodegenerative manipulations in these regions (43). Similarly, reinnervation following MA neurotoxicity may be responsible for increases in 5-HT availability (17). The present study shows that MPFC and CdN 5-HT levels increased over time, but 5-HT in the HPC remained at 30% of control levels. Perhaps a time course in the current study longer than 237 days would have revealed increases in HPC 5-HT levels, as several studies have suggested slower reinnervation takes place in the HPC (14,36). Nonetheless, because there is no absolute increase of 5-HT in the HPC, perhaps other mechanisms are also involved. Examination of turnover rates within the HPC using 5-HIAA/5-HT ratios suggests that changes in surviving 5-HT terminals augment metabolic activity by 237 days. Although further research is necessary, this enhancement in turnover may be the basis for a mechanism of recovery in the absence of sprouting.

Behavioral/Biochemical Relationship

At 65 days postinjection MA animals were impaired on the second block of trials on day 1 of the Morris water-maze place task. This suggests that MA-treated animals are initially impaired at encoding spatial cues or utilizing spatial knowledge. Later, these same animals displayed no impairments a) following a change in platform position on block 6, which took place after 20 trials; b) on the moving platform task, which involves learning a new platform position each day for 12 days; and c) in forming associations for novel spatial cues after 80 swim trials in a different training environment. In the present study we find no robust spatial deficits in MA-treated animals, and those subtle aberrations that exist likely undergo recovery.

The reason for this recovery is an important question to answer and the present data suggest several possibilities. First, recovery from the initial deficit in escape latency was observed following extensive experience with the water maze. This means that such deficits produced by MA neurotoxicity may be overcome with training. The other possibility is that compensatory changes in monoamine levels may provide a significant contribution to behavioral recovery after a long interval following neurotoxic exposure to MA.

Recovery in tissue content of monoamines, especially 5-HT, could underlie the behavioral improvement. In the present study significant increases were found at 237 days relative to measures taken on day 48. Specifically, increases in CdN DA and 5-HT, and HPC 5-HIAA were observed. It is suggested that these biochemical increases may reflect the compensatory mechanisms that could lead to behavioral improvement.

DA and 5-HT were reduced at 48 days post-MA treatment, yet both showed some recovery later. Further, there ap-

pears to be a differential rate of recovery insofar as CdN DA showed significant levels of recovery at 139 days, whereas 5-HT showed no recovery until later and not in all brain structures studied. The interval when DAergic changes were measured spans the time profile for behavioral changes, suggesting that this specific neurochemical change could underlie behavioral recovery.

The idea that changes in DA function may be important in water-maze performance is supported by much evidence. DA depletions produced by 6-hydroxydopamine reliably impairs Morris water-maze performance (10,41). Although the magnitude of the deficits in swim performance was more extensive in the above studies compared to the present experiment, their CdN DA depletions were also more severe ($\geq$67 vs. 59% at 48 days in the present study).

It has been suggested that deficits in water-maze performance following DA denervation may be due to motor problems (33). Motor impairments following MA treatment have also been attributed to poor motor initiation on a reaction time task (26) and in balance beam and active avoidance tasks (39). Therefore, it is reasonable to suggest that the deficit observed here is due to loss of DA innervation specifically. Further, the recovery from this deficit parallels an increase in CdN DA levels, supporting this idea. This effect is akin to improvement in balance beam and active avoidance tasks produced by L-dopa treatment following MA (39).

In contrast, large 5-HT depletions have not been shown to impair place task performance [see (19) for review]. For example, 70% reductions in cortical or hippocampal 5-HT by the neurotoxin 5,7-dihydroxytryptamine does not affect place learning (20,27). In the present study, no recovery was observed of 5-HT levels between 48 to 139 days in MPFC and CdN, or in HPC at any time. Further, increases in HPC 5-HIAA turnover are unlikely to have played a major role in water-maze performance recovery because this occurred much later. In summary, no major role for 5-HT in recovery of place task performance is suggested.

The two issues of extensive training and neurochemical recovery raised as putative explanations for improvement in the place task may also account for the intact performance by MA-treated rats in the place task at 165 days. Animals tested on the place task at 165 days had extensive experience utilizing spatial information in previous T-maze testing. Further, the increase in postmortem CdN DA observed in one group of animals assayed at 139 days may have been present in those animals tested at 165 days in the place task. The possibility that training and biochemical recovery may have alleviated an acquisition impairment is a strong possibility.

Finally, it has been argued that the behavioral ability of MA-treated rats is relatively unimpaired due to presynaptic changes that normalize synaptic levels of DA (29). Unfortunately, only pharmacologically evoked locomotor activity in the form of 90° turns has been investigated (30). It is important to characterize in a more neurologically complete manner how changes in DA activity may impact the sparing/recovery of behavior. The present data extend the previous results of Robinson et al. (29) by demonstrating a mild deficit in the initial stages of acquiring a learning set in spatial navigation; and this may occur at a time when presynaptic compensatory changes are also taking place. Thus, our current finding of an enhancement in CdN DA levels indirectly supports the hypothesis that normalized extracellular DA may contribute to the relatively intact behavioral ability of MA-treated animals.

In conclusion, significant biochemical depletions following MA-induced neurotoxicity undergo substantial recovery over time perhaps sufficient to maintain sparing and produce recovery of function. The impact and time course of persistent HPC 5-HT depletion remains to be fully disclosed.

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