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Effects of 3,3′-Iminodipropionitrile on Acquisition and Performance of Spatial Tasks in Rats

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LLORENS, J., K. M. CROFTON AND D. B. PEELE. Effects of 3,3′-iminodipropionitrile on acquisition and performance of spatial tasks in rats. NEUROTOXICOL TERATOL. 16(6) 583-591, 1994.—3,3′-Iminodipropionitrile (IDPN) has been reported to disrupt learning and memory in rats (24). The present work addressed the effects of IDPN on tasks requiring the use of spatial information. Separate groups of male rats were dosed with IDPN (IP, in 1 ml/kg saline) for 3 consecutive days and tested in the following procedures: (a) step-through passive avoidance conditioning (0, 100, 150, and 200 mg/kg/day); (b) Morris water maze (MWM) acquisition and retention (0, 125, 150, 175, and 200 mg/kg/day); (c) radial arm maze (RAM) acquisition (0, 100, 200, and 400 mg/kg/day); (d) RAM steady-state performance (0, 200, and 400 mg/kg/day); (e) repeated acquisition in the RAM (0, and 200 mg/kg/day). The vestibular toxicity of IDPN resulted in alterations in spontaneous behavior or swimming deficits in 5 of 8 rats treated with 175 mg/kg/day and in all the animals dosed with 200 or 400 mg/kg/day. IDPN increased step-through PA latencies at 200 mg/kg/day but not at lower doses. In the MWM, no performance deficits were observed at the dose levels preserving the swimming ability of the animals. In both the acquisition and the steady-state RAM tasks, IDPN (400 mg/kg/day) induced an increase in both choice errors and perseverative errors. In the RAM repeated acquisition paradigm, IDPN (200 mg/kg/day) induced performance deficits that included a decreased rate of within-session reduction in errors. The present data show that IDPN disrupts performance of tasks requiring spatial learning and memory and indicate that these deficits can be in part caused by an acquisition deficit.

INTRODUCTION

3,3′-IMINODIPROPIONITRILE (IDPN) produces a number of adverse effects on the nervous system (see 3,13,15). Perhaps the most widely characterized effect is the occurrence of swellings in the proximal portion of axons (4,5) due to an accumulation of neurofilament proteins consequent to deleterious effects of the chemical on their axonal transport (14). Evidence also exists indicating that IDPN affects several sensory systems including the visual (16,31), olfactory (10), auditory (6), and vestibular (20) systems. In the CNS, the giant axonal swellings have been seen at levels as high as the mesencephalon (4), but initial neuropathological assessments of IDPN-exposed rats failed to reveal evidence of neuronal degeneration (4,30). However, recent data indicate that the chemical may indeed cause significant neuronal degeneration in cortical areas (18).

Laboratory rodents exposed to IDPN display a syndrome of permanent abnormalities in spontaneous behavior. The IDPN syndrome is characterized by repetitive head movements, retropulsion, circling, and hyperactivity (6,7,17,33), and has been recently identified as a consequence of the vestibular toxicity of the compound (20). In addition to the sensory and motoric effects, recent evidence suggests that IDPN also may have deleterious effects on cognitive processing (24). In that study, a 3-day administration of IDPN produced a pro-

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found disruption of performance on a number of tasks requiring learning and memory at 4 weeks postexposure, a disruption that could not be attributed to effects on sensory and motor systems. Although Peele et al. (24) included tests as diverse as passive and active avoidance, and odor and flavor aversion conditioning, further studies are necessary to fully characterize the deficits induced by IDPN on learning and memory, and to determine the behavioral mechanisms responsible for these deficits.

The aim of the present work was to study the effects of IDPN on acquisition and performance of tasks requiring learning and memory of spatial information, using the Morris water maze (MWM; 21,22), and the 8-arm radial arm maze (RAM; 23). Both procedures are widely used in the study of spatial learning and memory and have been shown to provide useful animal models of the behavioral consequences of neurotoxic insults (e.g., 34,35). Acquisition of performance after IDPN exposure was studied in both the MWM and the RAM. In addition, the effect of IDPN on RAM steady-state performance (e.g., 25) was also studied. To determine the contribution of deficits in acquisition of new behavior in the impairments observed, the effects of IDPN on a repeated acquisition task (1,2) were studied as described by Peele and Baron (26) for the RAM. Finally, an assessment of passive avoidance (PA) was included in the present experiments to compare dose–response relationships with those of previous work (24).

METHOD

Animals

Male Long-Evans hooded rats (Charles River Breeding Co., Raleigh, NC) were obtained at 60 days of age and maintained in a colony certified by the American Association for Accreditation of Laboratory Animal Care. The animals were housed in standard acrylic hanging cages and maintained on a 12L:12D cycle (0600:1800 h). Temperature was maintained at 21.0 ± 2°C and relative humidity at 50 ± 10%. A minimum of 7 days following arrival was provided to the animals for acclimation. Rats in the MWM and PA experiments were housed two per cage with standard food pellets (Purina Lab Chow) freely available. For the RAM experiments, rats were housed 1 per cage, and maintained at 350 g of body weight by limiting their daily food ration. Tap water was provided ad lib.

Dosing

Rats were injected IP on 3 consecutive days with 1 ml/kg of either saline vehicle or a mixture of saline and IDPN (Eastman-Kodak Co., Rochester, NY) in dosages of 100, 125, 150, 175, 200, or 400 mg/kg/day, for cumulative dosages of 0, 300, 375, 450, 525, 600, or 1200 mg/kg. Previous studies indicated that no lethality was associated with these dosing regimens (6,18).

Apparatus

Passive avoidance (PA). The apparatus used for PA conditioning has been described elsewhere (24). Briefly, it consisted of a shuttle box equipped with a door to restrict access between equal-sized illuminated and dark compartments. The grid floor was connected to a shock source for shock delivery during training.

Morris water maze (MWM). The apparatus consisted of a circular pool, 148 cm diameter × 60 cm high, with a water depth of 40 cm. An escape platform, 10 cm in diameter, was placed 1.5 cm beneath the surface in a fixed location. Further details have been reported elsewhere (19).

Radial arm maze (RAM). An automated version of the RAM was used (25,26). Briefly, the maze consisted of eight alleys radiating from a central arena. Access to the arms was controlled by guillotine doors; photodiodes detected the position of a rat in the maze. Food delivery to a cup at the end of each alley was performed by means of automatic pellet dispensers. Stimulus conditions and data collection were arranged by a minicomputer and SKED-11 software (28).

Procedures

Passive avoidance. Animals (n = 10/group) were given 0, 100, 150, or 200 mg/kg/day of IDPN and tested 4 weeks after dosing. PA conditioning was assessed as described in detail elsewhere (24). A 0.5-ma 0.5 s foot shock was delivered when the rat entered the dark compartment during the training session. The test session took place 24 h later. An upper limit of 600 s was imposed on step-through latencies.

Morris water maze. Animals (n = 8/group) were given 0, 100, 125, 150, 175, or 200 mg/kg/day of IDPN. Doses were chosen on the basis of previous work indicating deficits in PA conditioning at doses as low as 133 mg/kg/day (24), and knowledge that a 400 mg/kg/day dosing regimen may cause a swimming deficit (see also 33). Rats were tested in the MWM (21,22) as described in detail elsewhere (19). The animals were given twenty 1-min trials on days 18 to 22 after the last dose. On the first trial, rats were rated as either able or unable to swim. Inability to swim was easily observed as the animal would quickly sink after being gently placed in the water. Animals unable to swim were immediately removed from the water and no longer tested. Latencies to reach the escape platform were obtained for the remaining animals. A free swim test, in which the escape platform was removed and the time spent in each quadrant of the pool was recorded for 1 min, was conducted 2 h after the last acquisition trial. Animals in this MWM experiment were weighed regularly to determine the effects of IDPN on body weight.

RAM acquisition. After 4 weeks on food restriction, animals (n = 5/group) were given IDPN (0, 100, 200, or 400 mg/kg/day). Three stages of training took place two weeks later. First, rats were confined in one maze arm with several food pellets placed in and around the food cup. In addition, food pellets were dispensed for each nose-poke into the food cup until 25 pellets were delivered. In the second stage, rats were confined in one of the maze arms in which each nose-poke delivered one pellet to the food cup until 25 pellets were delivered. The arms used for the different pretraining stages were changed from session to session. The acquisition experiment began 5 weeks after treatment and after all rats successfully completed the pretraining tasks. Rats were studied for 39 sessions arranged once a day (5 days/week) before feeding time. Each session was performed according to Peele and Baron (25), except that the inter-selection interval was 5 s. Briefly, pellet delivery was contingent on selection of an arm not selected previously. Sessions were terminated when all 8 pellets were collected or after a 1200 s upper limit (excluding inter-selection interval time) was reached.

RAM steady state performance. A separate group of animals showing criterion performance in the RAM standard procedure described above (25) were selected to study the effects of IDPN on steady-state performance. The animals had been used for drug studies, and had 50–60 sessions training. All the drugs used had only acute effects on performance with
no evidence of residual effects. The animals received saline vehicle \((n = 9)\), \(200 (n = 8)\), or \(400 (n = 9)\) mg/kg/day of IDPN, with treatments assigned according to performance accuracy to balance groups. Vehicle or IDPN solutions were given at the end of three consecutive experimental sessions. Performance of the rats was assessed up to 20 sessions after IDPN treatment.

Repeated acquisition in the RAM. The repeated acquisition procedure developed by Peele and Baron (26) was used. Rats were first given pretraining and training in the standard RAM procedure as just described (25), after which the repeated acquisition task was introduced (sessions 1–70). The experiment was terminated after session 70 due to a viral infection (rat siaoadacronymis/corona virus) in the animal colony. This virus causes a temporary infection of the salivary glands and in advanced stages infects the tear ducts of the eye. We had no evidence that any animals used in this experiment were infected (only a very small percentage of animals in the colony were infected), and consider it unlikely that this event affected the animals in the present study. However, the sacrifice of the entire animal colony was required to prevent further infestation and to allow sterilization of the facility. In brief, each experimental session consisted of an acclimation period (60 s), and 14 trials in which a constant set of 4 arms was baited. Trials terminated after the rat obtained all four pellets or after 400 s elapsed. Trials were separated by a 5-s intertrial interval. Rats were given saline vehicle \((n = 6)\) or 200 mg/kg/day of IDPN \((n = 8)\) after sessions 43 to 45. The 200 mg/kg/day dose level was chosen because it produces less clinical signs compared to the 400 mg/kg/day dosage.

Data Analysis

Passive avoidance. Step-through latencies were analyzed by Kruskall-Wallis analysis of variance (ANOVA), with pairwise comparisons analyzed with Wilcoxon's test.

Morris water maze. Body weights were analyzed by repeated measures MANOVA, with time as the within-subject factor. Latency data from the 20 acquisition trials were analyzed by repeated measures MANOVA, with both trial and day as within-subject factors. Orthogonal contrasts were used for further comparisons. The percentage of time spent during the free swim test in the quadrant where the escape platform had been located was analyzed by one-way ANOVA.

RAM acquisition. The number of sessions to reach a learning criterion of two consecutive sessions without an error was used for statistical analysis. Total errors (selection of an arm already visited during the session), and perseverative errors (selection of the arm selected in the previous choice) were analyzed by the Lifetest procedure (27), nonparametric analysis of variance corrected for right censored observations (i.e., animals that did not reach criterion at the end of the experiment). The same procedure was used for pairwise comparisons.

RAM steady state performance. The number of errors per session and the percentage of perseverative errors were averaged across sessions and analyzed by one-way ANOVA, followed by Duncan's test for posthoc analysis.

RAM repeated acquisition. The number of correct and incorrect arm selections, the session time, and the number of pellets obtained were recorded. In addition, an index-of-curvature statistic (9) was computed. This index was used to describe the decline of within-session errors. Index values approaching \( -1.0 \) indicate a decline in within-session errors, whereas values approaching zero indicate a constant error rate across trials. The data from the repeated acquisition experiment were analyzed by repeated measures ANOVA, adjusted according to the Geisser-Greenhouse solution, followed by orthogonal contrasts for posthoc analysis.

All statistical analysis were performed using the SAS program package (27).

RESULTS

General Observations

The effects of IDPN (0, 100, 125, 150, 175, or 200 mg/kg/day) on body weight are shown in Fig. 1. There was a significant day × treatment interaction, \(F(80, 134) = 2.35, p < 0.0001\). Significant effects of treatment were found for days
3-19 [all Fs(5, 42) > 2.53, ps < 0.05]. On these days, there were reduced body weights in the rats dosed with either 175 or 200 mg/kg/day of IDPN, except for Day 3, for which only the high dose group was affected. No body weight data are available for the groups of animals in the RAM experiments due to the animals being on a food restriction paradigm.

IDPN induced a dose-dependent impairment in swimming ability. Table 1 shows the number of rats affected in each dose group. Animals displaying deficits in swimming, as well as animals treated with 200 or 400 mg/kg/day of IDPN and not tested for swimming ability, showed the characteristic behavioral syndrome including repetitive head movements, retropulsion, circling, and hyperactivity (6,7,20,33). No symptoms were observed in the rats dosed with 100, 125, or 150 mg/kg of IDPN for 3 days.

### Passive Avoidance

The effects of IDPN on PA conditioning are shown in Fig. 2. Whereas no differences were observed among dose groups during training, IDPN had a significant effect on median test latencies ($\chi^2 = 10.10, 3 \text{ df}, p < 0.05$). Posthoc comparisons revealed that the 200 mg/kg/day group was different from controls ($z = 2.63, p < 0.05$), and that the 100 and 150 mg/kg/day groups were not ($p$ values > 0.6).

### Morris Water Maze

Data from the MWM are shown in Fig. 3. Analysis of the acquisition trials (3A and B) revealed significant day, $F(4, 27) = 30.45, p < 0.001$, trial, $F(3, 28) = 9.01, p < 0.001$, and treatment, $F(4, 30) = 4.83, p < 0.01$, effects, although no interactions were significant ($p$ values > 0.4). Posthoc analysis revealed that animals in the 175 mg/kg/day group ($n = 3$) showed longer latencies than controls, $F(1, 9) = 16.5, p < 0.01$. No effects of the IDPN treatment were obtained for the dose groups with intact swimming ability. When the escape platform was removed (free swim), animals in all dose groups showed a similar preference for the quadrant where the platform had been located previously (Fig. 3C, $p > 0.8$).

### RAM Acquisition

Control animals rapidly acquired the RAM task, and the number of errors dropped to 0-2 per session within 15-20 sessions (Fig. 4A). Although there was an apparent slowing of task acquisition in the 200 mg/kg/day group (Fig. 4A), the effects of IDPN ($\chi^2 = 10.8, 3 \text{ df}, p < 0.05$) were only reliable when comparing control versus the 400 mg/kg/day animals ($\chi^2 = 8.33, 1 \text{ df}, p < 0.01$). No differences were obtained when comparing control animals with those treated with 100 or 200 mg/kg/day of IDPN ($\chi^2$ < 1.4, $p > 0.2$). Note that none of the 400 mg/kg/day animals ever reached criterion performance (Fig. 4A, inset). In contrast, criterion performance for the percentage of sequential selections of the same arm ("perseverative" errors) was reached by all the animals (Fig. 4B). Control animals soon avoided, and after 13 sessions made no more, errors of this kind. Animals treated with IDPN

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**TABLE 1**

**LOSS OF SWIMMING ABILITY AFTER IDPN EXPOSURE**

| Dose (mg/kg/day, 3 Days) | Unable to Swim/Total in Group |
|-------------------------|------------------------------|
| 0                       | 0/8                          |
| 100                     | 0/8                          |
| 125                     | 0/8                          |
| 150                     | 0.8                          |
| 175                     | 5/8                          |
| 200                     | 8/8                          |

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**FIG. 2.** Effects of IDPN on PA conditioning. Data are median (+ interquartile range) step-through latencies during training and testing ($n = 10$/ group). An upper limit of 600 s was imposed on step-through latencies. The median test latency for the 200 mg/kg animals was significantly different ($p < 0.05$) from the control median latency.
FIG. 3. Effects of IDPN on acquisition and retention of the MWM task (n = 8/group, except for the 175 mg/kg/day group, for which n = 3). The latencies to find the submerged platform were pooled by day (A), and by trial (B). The symbol legend in Panel B applies to both A and B. Panel C shows the time spent, when the platform was removed for the 21st trial, in the quadrant where it had been located during the acquisition trials.

FIG. 4. Effects of IDPN on acquisition of the standard RAM task (n = 5/group). Session number 1 took place 5 weeks after dosing. (A) Each point represents the mean number of choice errors (i.e., visits to arms already selected during the same session). Inset shows number of sessions required to reach the learning criterion of two consecutive sessions with no choice errors. (Note: none of the 400 mg/kg/day animals ever reached criterion performance). (B) Each point represents the mean percentage of perseverative errors (i.e., selecting the arm entered in the previous choice). Inset shows number of sessions required by the rat to reach the learning criterion of two consecutive sessions with no perseverative errors. *Significantly different from control median (p < 0.05).
also reduced the number of perseverative errors (Fig. 4B, inset), but the rate of reduction was much slower compared to controls as evidenced by group differences in the number of sessions to criterion for perseverative errors ($\chi^2 = 10.25, 3$ df, $p < 0.05$). Posthoc analysis indicated differences between the control and the high-dose group ($\chi^2 = 8.33, 1$ df, $p < 0.01$) but not between the control and the 100 or the 200 mg/kg/day groups ($\chi^2 < 1, p > 0.3$).

**RAM Steady State Performance**

The steady-state performance of animals in the RAM was also impaired by IDPN (Fig. 5). Whereas error rates remained in the 0 to 2 range for control animals, IDPN treatment induced an increase in this parameter [Fig. 5A; $F(2, 23) = 35.79, p < 0.0001$. Posthoc analysis revealed that animals treated with 400 mg/kg/day made more errors than did control animals. Unlike the control animals, who made no perseverative errors, IDPN-treated animals tended to select an arm that had just been visited (Fig. 5B). This tendency was most evident during the early sessions (i.e., 3–11), with recovery evident afterwards. Analysis of the mean percentage of sequential selections of the same arm (Fig. 5B, inset) showed that the effect of IDPN, $F(2, 23) = 8.22, p < 0.01$, was significant for the 400 mg/kg/day group.

**Repeated Acquisition in the RAM**

Performance quickly improved during the acquisition phase (i.e., trials 1–20) of the repeated acquisition paradigm (Fig. 6). Just prior to IDPN administration (sessions 43–45), animals were making an average of only 10 errors per session (Fig. 6A), and completed the task in around 200 s (Fig. 6B). Whereas control animals maintained a similar level of performance, treatment with 200 mg/kg/day IDPN profoundly impaired performance. The number of errors increased (Fig. 6A), as also did the session times (Fig. 6B). Significant treatment by session interactions were obtained for both the number of errors and the session time, $F(65, 780) = 4.32, p < 0.001$, and $F(65, 780) = 4.81, p < 0.01$, respectively. Some IDPN rats were unable to complete the task from sessions 44 to 59, as evidenced by a decrease in the number of correct choices (Fig. 6C); performance improved thereafter, and all IDPN animals collected the 56 food pellets on the session number 60 and on most of the remaining sessions. However, the improvement in the number of errors and session time did not reach control levels of performance by session 70.

The rate of within-session decline in errors, as measured by the index-of-curvature statistic, is shown in Fig. 6D. Session-by-session posthoc analysis, following a significant session-by-treatment interaction, $F(65, 780) = 2.40, p < 0.05$, showed that IDPN impaired the rate of reduction in the number of errors across trials within a session.

**DISCUSSION**

IDPN exposure resulted in body weight loss, alterations in spontaneous behavior, and swimming deficits. Rats exposed to IDPN also had deficits in PA conditioning. Performance in a standard 8-arm RAM was impaired by IDPN in both the acquisition and the steady-state performance paradigms. In both these paradigms, IDPN increased the number of total errors, as well as the perseverative errors. When tested in a repeated acquisition paradigm in the RAM, IDPN-treated rats showed performance deficits that included a decreased rate of within session reduction in errors.

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**FIG. 5.** Effects of IDPN on RAM performance once the animals had acquired steady-state performance ($n = 9$ for the saline and 400 mg/kg/day groups; $n = 8$ for the 200 mg/kg/day group). (A) Each point represents the mean number of errors per session during the three dosing sessions and the 20 postdosing sessions. (B) Each point represents the mean percentage of perseverative errors (i.e., visits to arms already selected during the same session). Inset shows mean number of errors per session during the three dosing sessions and the 20 postdosing sessions. *Significantly different from control mean ($p < 0.05$).
The effects of IDPN on body weight, spontaneous behavior, and swimming ability have been known for a number of years (7,29,33). The mechanisms by which IDPN reduces body weight include reduced food and water intake (29). The alterations in both spontaneous behavior and swimming have been recently attributed to the vestibular toxicity of IDPN (20). Dose-response relationships for these types of behavior are in good agreement with previous data from our laboratory (6,18,24). Also, the swimming deficits appeared at the same doses (175 mg/kg/day) that modify body weight and spontaneous behavior.

The PA experiment was included in the present study to allow direct comparison with previous data on the effects of IDPN on performance of tasks requiring learning and memory. In the present work, IDPN was found to increase PA step-through test latencies, consistent with the results by Peele et al. (24). However, differences in effective doses were found, because PA conditioning deficits had been reported to occur at doses of IDPN as low as 133 mg/kg/day (24). Whereas no explanation is available for this discrepancy, the present work found deficits in PA conditioning at the same dose levels that modify spontaneous behavior, so the hypothesis that IDPN induces learning deficits at doses below those modifying motor behavior was not confirmed.

The swimming deficits induced by IDPN precluded the use of the MWM to study its effects on this performance for doses greater than 175 mg/kg/day. Although 100, 125, or 150 mg/kg/day of IDPN had no effect on behavior in the MWM, the effect of 175 mg/kg/day on escape latencies may be explained by impairment of swimming behavior. First, only 3 out of 8 animals in the 175 mg/kg/day group could be tested because of swimming deficits. Second, both the free-swim test and the interaction statistics for acquisition showed no difference between the control and the 175 mg/kg/day animals. So, whereas no effects on MWM behavior were observed at doses of IDPN that did not impair swimming, swimming deficits confounded the use of the MWM to study spatial performance in animals exposed to higher doses.

In contrast to the MWM, the effects of IDPN on motor behavior did not impede the use of the RAM to study acquisition and performance of spatial tasks. Rats treated with 200 or 400 mg/kg/day of IDPN were still able to enter the arms and to collect pellets in the RAM. In the acquisition experiment, animals treated with 400 mg/kg/day of IDPN failed to reach criterion performance for total errors by the end of the experiment. These data may be explained by a failure in either: (a) emitting the appropriate responses (e.g., hyperactivity may have impeded the animals to select arms); (b) acquiring the appropriate behavior (e.g., a deficit in acquiring the test rules, constant across sessions); or (c) using session-specific informa-
tion (i.e., the list of arms already visited). The sequential selections (perseverative errors) data indicated that animals treated with IDPN were eventually able to reach criterion performance levels but need more sessions to reach the learning criterion. Rats treated with IDPN required more training sessions than control animals to reach criterion performance in a PA task (24). In both cases, an ability to emit the required behavior was shown, thus suggesting the involvement of acquisition deficits in the behavioral effects of IDPN.

A further characterization of the IDPN-induced deficits in RAM performance was provided by the steady-state study. In this design, IDPN disrupted the RAM performance of animals that had acquired stable performance prior to dosing. These effects of IDPN in the RAM steady-state experiment could not be due to an interference with the across-session acquisition of appropriate responses. As in the acquisition experiment, whereas the frequency of total errors remained roughly stable (Fig. 4A and 5A), perseverative errors tended to remit (Fig. 4B and 5B). The similarity between the shapes of the perseverative errors function in the acquisition and the steady-state performance RAM experiments would be easily explained by the hypothesis that they reflect a behavioral recovery: the animals in the acquisition experiment were dosed 5 weeks before the first session (Fig. 4), whereas the animals in the steady state experiment where dosed after sessions 1 to 3 (Fig. 5). The occurrence of perseverative errors also in the steady-state paradigm suggest that behavioral disruption (e.g., difficulties in pellet collection because of deficits in sensory or motor function) may have a role in the IDPN-induced deficits in RAM performance. The deficits induced by scopolamine in steady-state RAM performance do not involve perseverance errors (25). However, the hypothesis of behavioral disruption does not rule out concomitant cognitive deficits in the effects of IDPN on RAM performance.

The data from the repeated acquisition experiment provided evidence that acquisition deficits may indeed be involved in the performance effects induced by IDPN. The motoric competence required by this task is the same as that required by the standard RAM task. However, it includes more demands on learning and memory capacities, because three levels of information must be used by the animal: (a) information relative to the test (constant across sessions); (b) the list of baited arms (constant within the 14 trials of a session but varying from session to session); (c) trial-specific information (i.e., the arms already visited within a trial). The sensitivity of repeated acquisition paradigms to toxicant-induced deficits in learning and memory has also been demonstrated in other behavioral models (e.g., the MWM, 34). As in the RAM acquisition and steady-state paradigms, the repeated-acquisition paradigm indicated that IDPN impairs performance (Fig. 6). An increased number of errors was still present after the animals recovered their ability to complete the task. In addition, in the repeated acquisition paradigms, acquisition may be defined as the rate of within-session decline in errors as reflected by negative index-of-curvature values. The use of the index-of-curvature statistic seems to be quite useful in quantitative assessments of behavioral acquisition as well as treatment effects on acquisition (26,32). So the significant increases in the index-of-curvature values in the IDPN-treated animals (Fig. 6D) provided direct evidence that IDPN did in fact impair the ability of the animals to reduce the number of errors across trials within a session, indicating the presence of a deficit in acquisition.

The present data thus suggest that IDPN impairs RAM performance by inducing both a behavioral disruption as well as an acquisition deficit. The toxicity of IDPN on a number of sensory systems, and its consequences on motor behavior (see Introduction), may well be responsible for the performance deficits that seem to be related to behavioral disruption. The fact that the acquisition deficits revealed by the increases in the index-of-curvature were observed at doses inducing changes in motor behavior suggest that the sensory toxicity of IDPN may be also responsible for these deficits. To our knowledge, it is not known whether sensory deficits alone may induce acquisition deficits in the repeated acquisition in the RAM. However, a case could be made that mechanisms other than sensory toxicity may contribute to the observed IDPN-induced deficits in acquisition. First, IDPN has been shown to cause deficits in amygdala kindling development (11), an experimental model of synaptic plasticity (8,12). Second, IDPN induced a delay-dependent deficit in a flavor aversion conditioning paradigm for which intact performance was demonstrated (24). Both the CNS injury caused by IDPN (18), and its effects on axonal neurofilament transport (14) might have a negative impact on synaptic plasticity leading to learning and memory deficits. Additional research is clearly required to determine the relative contribution of the different actions of IDPN to the observed deficits in acquisition.

In summary, the present study addressed the effects of IDPN on acquisition and performance of tasks requiring spatial learning and memory. Whereas no deficits were detected in the MWM at doses that did not affect swimming ability, profound performance deficits were found in the radial arm maze tasks. The data from three different RAM tasks (acquisition, steady-state performance, repeated acquisition) suggested that both behavioral disruption and acquisition deficits may contribute to the effects of IDPN on RAM performance. Additional work is required to determine the contribution of the known toxic actions of the chemical (sensory toxicity, neuronal degeneration in the CNS, effects on axonal transport) to the observed deficits.

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