Proc. Natl. Acad. Sci. USA
Vol. 95, pp. 11435–11439, September 1998
Neurobiology

Immediate and long-term effects of neonatal MK-801 treatment on nonspatial learning

GRACE S. GRIESBACH AND ABRAM AMSEL

Department of Psychology and Institute for Neuroscience, University of Texas, Austin, TX 78712

Contributed by Abram Amsel, July 10, 1998

ABSTRACT **These experiments observed the immediate and long-term effects of neonatal treatment with MK-801 on patterned single alternation (PSA), a form of nonspatial, memory-based learning. Rat pups were injected daily on postnatal days (PND) 7–19, with MK-801 (MK^+) or the less active isomer of MK-801 (MK^-) (0.25 mg/kg), and trained at either PND 22 or 60. Rats treated with MK^+ or MK^- and trained on PND 22 were significantly impaired in PSA when compared with the saline control. Beyond the learning impairment, MK^+ rats showed an overall decreased running speed during training. They also presented an array of abnormal behaviors and significant weight loss. These nonassociative variables were determined for several doses (0.025, 0.05, 0.1, 0.15, and 0.20 mg/kg) through PND days 22–25. Rats that received the threshold dose for secondary effects (0.025 mg/kg) also showed an overall decrease in running speed, but failed to show a significant nonspatial learning impairment on PSA. The PSA learning impairment was found to be not long lasting; rats trained at PND 60, after neonatally receiving the original high dose of MK-801, did not differ from controls.**

The *N*-methyl-D-aspartate (NMDA) receptor/channel is involved in developmental changes, such as neuronal migration, survival, and the establishment of appropriate connectivity (1–3). Furthermore, it has been shown that postnatal NMDA blockade has an array of effects that reflect the role of the NMDA receptor during development (4–6).

It is well known that rats under the effects of MK-801, a noncompetitive NMDA receptor antagonist, show impairments in spatial (e.g., see refs. 7 and 8) and nonspatial learning (9, 10). It has also been shown that neonatal MK-801 administration leads to long-lasting alterations of body weight and motor behavior (11), as well as long-lasting effects on the NMDA receptor/channel function (12). Furthermore, Gorter and de Bruin (13) found that rats neonatally treated with MK-801 showed a spatial impairment as adults.

Long-term learning deficits and changes in the NMDA receptor/channel function have caused concern over the use of MK-801, not only during human infancy, but also during the third trimester of pregnancy, as implied by rat studies in which the first postnatal days (PND) correspond roughly to the third trimester of human gestation (14). These experiments described here tested for an impairment in patterned single alternation (PSA), a form of nonspatial learning, after neonatal treatment with MK-801. PSA is a memory-based discrimination task in which the discriminative cue is the memory from the previous trial outcome. Learning this task is, therefore, highly sensitive to the intertrial interval (ITI). PSA, at longer ITIs, has been related in several of our experiments to hippocampal function (e.g., see refs. 15 and 16). Because of the high density of NMDA receptors in the hippocampus, it is reasonable to expect that MK-801 will have an effect on its function.

In these experiments, preweanling pups were chronically exposed to MK-801 from PND 7–19, a period in which NMDA receptor sensitivity and subunit composition differs substantially from those of adults (17). The treated animals and controls were trained on PSA at one of two different ages to test for immediate or long-term effects on nonspatial learning. Both groups were trained with a 60-s ITI, the ITI at which deficits were observed after hippocampal lesion (15, 16). Our hypothesis is that, because of its crucial role in development, NMDA neonatal blockade will have behavioral consequences when tested at a later age.

Experiment 1

In the first experiment, pups were treated with MK-801 from PND 7–19 with the same dose that had previously been shown to cause long-lasting spatial learning impairment and changes in the NMDA receptor function (12, 13). Behavioral testing was performed from PND 22 to PND 25.

Methods. *Subjects and treatment.* The subjects were 24 male Sprague–Dawley rats, born and raised in our laboratory (Animal Resources Center, University of Texas at Austin). On PND 3, litters were culled to eight pups (day of birth is PND 0). The pups were assigned to one of three groups and were injected intraperitoneally, on postnatal days 7–19, with either 0.25 mg/kg MK-801 (MK^+ group, $n = 8$), 0.25 mg/kg of the less active isomer of MK-801 (MK^- group; $n = 8$), or saline (SAL group, $n = 8$). The MK-801 isomer has a high dissociation index and a low affinity to the NMDA receptor. Injections were given at 9 a.m. and 7 p.m. The SAL subjects were injected with an equal volume of saline vehicle. On PND 21, pups were weaned and single-housed. They were trained starting at PND 22 and food-deprived 24 hr before training.

Behavioral apparatus and procedure. A Plexiglas runway was used for the PSA training (Fig. 1). It consisted of a $13 \times 7.5 \times 12$ cm startbox, a $60 \times 7.5 \times 12$ cm runway, and a $17 \times 7.5 \times 12$ cm goalbox. A clear Plexiglas $12 \times 12 \times 12$ cm holding box was used between trials. The alley was divided by photocell circuitry into three 20-cm segments, which provided the measures of the approach responses in terms of start, run, goal, and total times (converted to speeds). Odors were expelled from the runway by means of a fan (115 V, 50 Hz, 10 cm in diameter) mounted on the rear wall of the goalbox. The outer walls of the apparatus and the holding box were covered with black cardboard and enclosed with clear hinged Plexiglas lids.

Rats were goalbox trained approximately 12 hr before PSA training. Each rat was placed in the runway apparatus with all doors open and allowed to explore for 15 min. The rat was then confined to the goalbox for 10 min and allowed to consume two pellets that were preplaced in the reward cup.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: NMDA, *N*-methyl-D-aspartate; PSA, patterned single alternation; PND, postnatal days; ITI, intertrial interval; R, rewarded; N, nonrewarded.

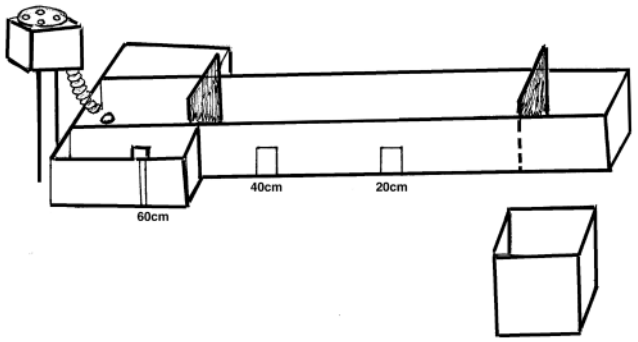

FIG. 1. Straight runway apparatus used for PSA training. Photocells are used to record running times.

From PND 22 to 25 rats were trained on PSA. Training consisted of 10 sessions, three sessions per day for 3 days and one session on the fourth day. Each session consisted of 40 trials, and training was at 60-s ITI. In PSA, rewarded (R) and nonrewarded (N) trials were single-alternated in the straight-runway apparatus. Therefore, 20-R and 20-N trials were presented in single alternation. On odd-numbered trials, the rat received a food pellet; on even-numbered trials, it was detained in the goalbox, without the pellet, for 30 s. Before and after each session the pup was weighed, and after the third session of each day it was postfed 15 pellets. Running times, converted to speeds, were recorded.

Results. The SAL group showed patterning from sessions 7–10. Rats treated with MK-801 did not discriminate between the R and N trials. The MK^- subjects also showed impaired discrimination, although not as severe as the MK^+ group. PSA training speeds for the MK^+, MK^-, and SAL groups were analyzed by means of a repeated measures ANOVA and are shown in Fig. 2 *A–C*). A significant main effect [$F(2,21) = 10.40$, $P < 0.001$] revealed differences among groups in overall running speed. The Bonferroni/Dunn control test showed that the MK^+ rats had significantly lower running speeds than the MK^- and SAL groups. Differences in discrimination learning are shown by means of a significant treatment × blocks × reward interaction [$F(78,819) = 2.84$, $P = 0.0001$]. This three-way interaction was analyzed further by pairing different treatments for comparison. All comparisons showed a significant treatment × blocks × reward interaction after Bonferroni correction: SAL vs. MK^+ [$F(39,546) = 4.46$, $P < 0.001$]; SAL vs. MK^- [$F(39,546) = 2.04$, $P < 0.01$], MK^- vs. MK^+ [$F(39,546) = 1.7$, $P < 0.05$].

Beyond the discrimination learning effects, subjects in the MK^+ condition showed increased reactivity to the environment, increased locomotor activity, and a 34% weight loss when compared with the SAL and MK^- groups.

Experiment 2

The purpose of the second experiment was to clarify the results from Experiment 1 by determining whether the severity of the learning impairment observed in the MK^+ rats was related to nonassociative variables, such as decreased running speed, during training and "secondary effects" observed after treatment. In addition, the aforementioned MK(−) subjects' behavior in Experiment 1 suggests that learning might be affected at a lower dose of MK-801 in the absence of any secondary effects. A threshold dose for secondary effects was obtained by observing activity, weight, and overall behavior at different doses, thus defining a low-dose control group with no secondary effects. These rats were then tested for PSA discrimination learning.

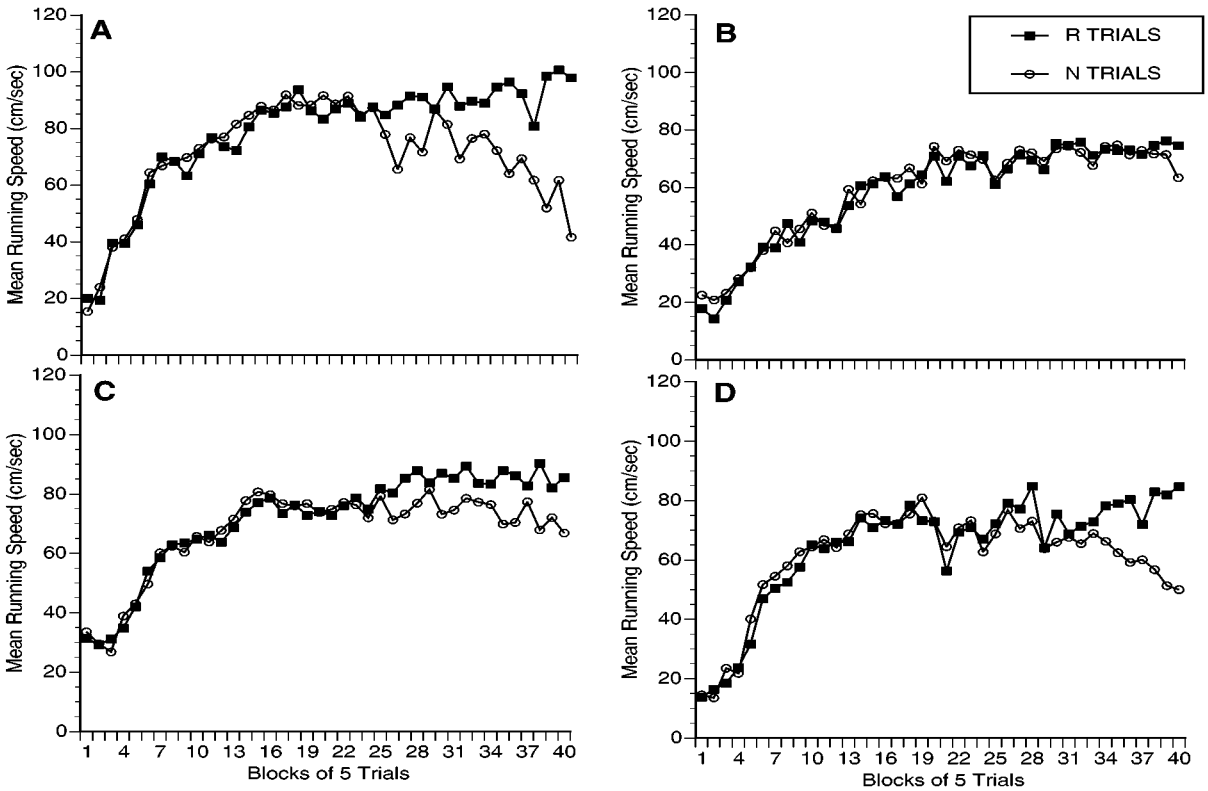

FIG. 2. Runway speeds over 10 sessions of PSA. Rats were trained from PND 22–25. (*A*) Saline control rats showing patterning from session 7. (*B*) Rats neonatally treated with 0.25 mg/kg of MK-801 unable to pattern and showing significantly lower overall running speed. (*C*) Rats neonatally treated with the less active isomer of MK-801 showing impaired patterning compared with saline control. (*D*) Rats neonatally treated with 0.025 mg/kg of MK-801 having significantly lower running speeds compared with saline control. These results did not differ significantly from the isomer condition.

Methods. *Subjects used for assessment of secondary effects.* Forty-two Sprague–Dawley rats, born and raised in our laboratory, were injected through PND 7–19 with MK-801 at different doses (0, 0.025, 0.05, 0.1, 0.15, and 0.25 mg/kg). Injections were given at 9 a.m. and 7 p.m. On PND 21 the pups were weaned and single-housed. Activity was assessed from PND 22 to 25.

Apparatus and procedures for activity assessment. Activity was observed with a nonmotorized wire-framed running wheel (16 cm in diameter) and a lat-maze, which consists of an alley surrounding a central box. The lat-maze was similar to one used by Sadile *et al.* (18). Lat-maze dimensions are illustrated in Fig. 3.

Activity in the running wheel and lat-maze was tested once daily for 4 consecutive days. Rats were placed in the running wheel and a photocell counted each revolution resulting from its movement. The number of revolutions was recorded at 5 and 15 min. Immediately after testing in the running wheel, rats were placed individually in the lat-maze for 5 min. Corner crossings, rearings, and groomings were recorded with a counter. The number of feces were counted at the end of the testing in the running wheel and the lat-maze. In addition, the experimenter recorded in a checklist any incidence of vocalizations, rotations, and biting.

Subjects for PSA training. The subjects were eight male weanling Sprague–Dawley rats from our breeding colony. The pups were injected intraperitoneally with 0.025 mg/kg of MK-801 (MK-low; $n = 8$). This was the dose in which no MK-801-induced secondary effects were observed. These animals were exposed to the same treatment schedule and training as in Experiment 1.

Apparatus and procedure for PSA. The runway apparatus was the same as in Experiment 1. Goalbox and PSA training schedules were also the same as in Experiment 1. Rats were trained on PSA from PND 22 to 25 with a 60-s ITI.

Results. *Weight.* A strong negative correlation was found between the MK-801 dose and weight ($r = -0.84, P < 0.0001$). A significant ANOVA [$F(7,40) = 16.80, P < 0.0001$] reflected that doses of 0.1 mg/kg and above were significantly lower from the saline control as shown through Bonferroni/Dunn weighing.

Activity assessment. No significant effect was found for the first 5 min of activity in the running wheel. The number of revolutions for total wheel time across sessions was analyzed through a repeated measures ANOVA. A significant main effect for dose [$F(6,34) = 3.025$, $P < 0.05$] was found. Bonferroni/Dunn analysis showed that a dose of 0.15 mg/kg was significantly different from the control (Fig. 4).

A significant day × dose interaction [$F(3,30) = 4.067$, $P < 0.03$] revealed that compared with the controls, a dose of 0.25 mg/kg decreased the number of corner crossings in the lat-box during the first day. No significant differences were found for grooming and rearing.

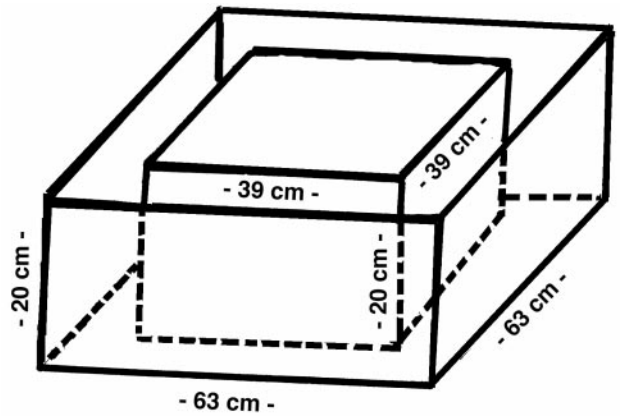

FIG. 3. Lat-maze apparatus.

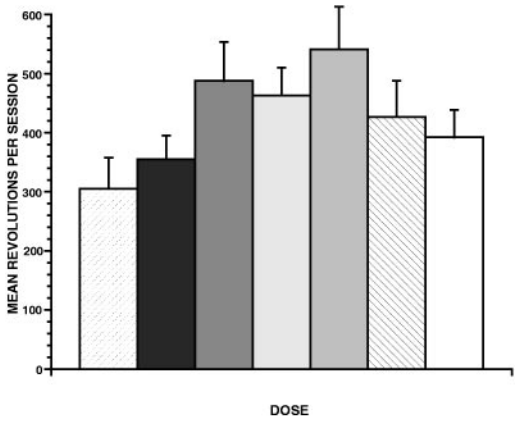

FIG. 4. Effects of neonatal MK-801 treatment on running wheel performance. Rats were dosed with 0, 0.025, 0.05, 0.1, 0.15, 0.2, or 0.25 mg/kg and tested at a weanling age. Data are average number of revolutions per 15 min. The significance of differences from the saline-treated group was determined through a repeated measures ANOVA followed by Bonferroni correction. *, $P < 0.05$

Incidences of vocalizations and of unprovoked biting of the experimenter were observed at doses of 0.05 mg/kg and above (Fig. 5). An ANOVA showed a significant main effect for biting [$F(6,34) = 2.75$, $P < 0.05$]. The Bonferroni/Dunn control test revealed that a dose of 0.15 mg/kg significantly increased biting. Circling, in both directions, was found only in doses above 0.15 mg/kg. There were no significant differences in the number of feces.

PSA. Being that no secondary effects were found with a dose of 0.025 mg/kg of MK-801, subjects receiving this dose were trained in PSA. Rats that received this lower dose of MK-801 did not show significantly impaired discrimination learning. However, significantly lower overall running speed was shown when compared with the SAL condition (Fig. 2*D*). This is supported by a repeated measures ANOVA that included the groups from Experiment 1 [MK-high (0.25 mg/kg), MK-low (0.025 mg/kg), SAL (saline) and MK^- (0.25 mg/kg of the isomer)]. (The "MK-high" group was previously described as "MK^+".) A significant three-way interaction of treatment × reward × blocks [$F(117,1053) = 2.40$, $P < 0.001$] was found. This three-way interaction was analyzed further by pairing different treatments for comparison. The following pairs had a significant treatment × reward × blocks interaction after Bonferroni corrections: SAL vs. MK-high [$F(39,546) = 4.46$, $P < 0.001$], MK-high vs. MK-low [$F(39,546) = 3.4$, $P < 0.001$], SAL vs. MK^- [$(39,546) = 2.04$, $P < 0.01$] and MK-high vs. MK^- [$(39,546) = 1.7$, $P < 0.05$]. Only a significant main effect was found when the MK-low was compared with the SAL

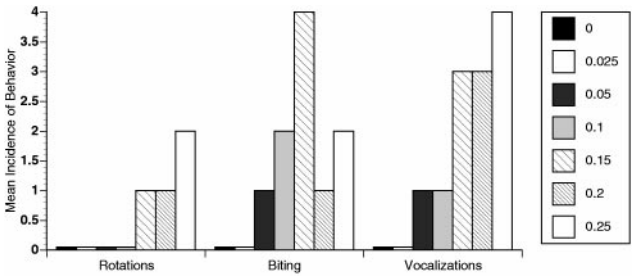

FIG. 5. Average incidence of biting, vocalizations, and circling during handling at a weanling age after MK-801 neonatal treatment with 0, 0.025, 0.05, 0.1, 0.15, 0.2, or 0.25 mg/kg. Circling was only found at doses above 0.15 mg/kg. Biting was significantly higher at 0.15 mg/kg.

condition [$(1,14) = 10.9$, $P < 0.05$], reflecting overall lower running speed. No significant differences were found between MK-low and MK^-.

For further analysis, running speeds from sessions 7–10, which correspond to the prediscrimination period, were examined. A significant main effect for treatment was observed [$F(3,27) = 8.65, P < 0.001$] in the prediscrimination phase. The Bonferroni/Dunn control test revealed that the SAL rats had significantly higher running speeds than the MK-high and MK-low groups, but not significantly higher than the MK^- rats. In addition, the MK^- group was significantly different from MK-high, but not from MK-low and SAL. Separate analysis of a discrimination phase revealed similar findings to the aforementioned three-way interaction analysis.

Experiment 3

Pups were treated from PND 7–19 with MK-801, as in Experiment 1, and trained on PSA from PND 60 to 86. The purpose of this third experiment was to determine whether the learning effect shown at an earlier age, as in Experiment 1, was long lasting.

Methods. *Subjects and treatment.* The subjects were 16 male Sprague–Dawley rat pups, born and raised in our laboratory. They were assigned to one of two groups and were injected intraperitoneally, on PND 7–19, with either 0.25 mg/kg MK-801 (MK^+ group, $n = 8$) or saline (SAL group, $n = 8$). Injections were given on the same schedule as in Experiments 1 and 2.

Behavioral apparatus and procedure. Rats were trained in a runway similar to the one used in the previous experiments, except that, because they were older, the overall length was 90 cm. The start, run, and goal segments were each 30 cm in length.

A week before training, rats were individually housed and placed on food deprivation, reaching an approximate 85% drop in body weight. Animals were also handled during this period. The feeding on the last day of deprivation consisted solely of 190-mg food pellets, which were used as rewards during runway training. Rats were goalbox trained approximately 12 hr before PSA training. Each subject was placed in the runway apparatus with all of its doors open and allowed to explore for 15 min. Then the rat was confined to the goalbox for 10 min and allowed to consume two pellets that were preplaced in the reward cup.

Training on PSA was from PND 60 to 86, and consisted of 1 session per day, a total of 26 sessions. Each session consisted of 24 trials. Animals were trained at a 60-s ITI, and there were 12-R and 12-N trials presented in single alternation. On the odd-numbered trials, the rats received a food pellet. On the even-numbered trials, the animal was detained in the goal box, without the pellet, for 30 s. Running times, converted to speeds, were recorded. Before each session the rat was weighed and after each session was postfed 13 g of rat chow 30 min.

Results. No significant differences were found among the SAL and MK^+ groups in PSA discrimination learning or running speeds.

Discussion

Experiment 1 showed that neonatal treatment with MK-801, at a dose of 0.25 mg/kg, severely impaired learning of a nonspatial nature in weanling rats in terms of a deficit in onset and magnitude of the PSA discrimination. It is unlikely that these results are a direct effect of MK-801, being that the time course of MK-801 binding does not exceed 150 min (19) and that the subjects were trained on the third day after receiving the drug treatment. Instead, as a consequence of MK-801 administration at a period in which the NMDA receptor is involved in developmental changes, the learning impairment suggests a structural alteration in neuronal development.

In addition to the learning impairment, anomalous behavior and significant weight loss were observed in subjects that received a relatively high dose of MK-801. These secondary effects are dose dependent and do not appear to have had a severe effect on learning. Although none of the secondary effects were observed in rats treated with the less active isomer of MK-801, discrimination learning was impaired. This learning impairment was not as severe as that observed after neonatal MK-801 treatment at a relatively high dose (0.25 g/kg). To reiterate, the data suggested that a lower dose of MK-801 might result in effects similar to those after isomer treatment. Although rats treated at the lower dose of MK-801 did not show a significant learning impairment in PSA, their overall running speed during training was significantly lower than the saline control. The fact that there were no significant differences between the MK^- and MK-low groups, in discrimination and running speeds, suggests that a low dose of MK-801 may affect learning in the absence of any secondary effects. It appears that NMDA receptor blockade during rat postnatal development has deleterious consequences on nonspatial learning in a dose-dependent manner. Furthermore, the overall running speed appeared to be dose dependent, in particular during the prediscrimination period in which the animals simply learn to run toward the goalbox. Although rats treated at a higher dose showed significantly slower running speeds, they could learn to run toward the reward.

The prediscrimination acquisition phase is comparable to a partial reinforcement acquisition schedule. The possibility that the decrease in running speeds shown by the MK-high group results from the rats reaction to nonreward would be a surprising result, being that a decrease in running speed has not been observed after teratogenic manipulations (20, 21). On the other hand, the absence of hypermotility is not surprising because no increase in motility was found in the running wheel or lat-maze at the higher dose (0.25 mg/kg) of MK-801. It would be interesting to know whether rats that received a dose that increased activity in the running wheel (0.15 mg/kg) would fail to show a decrease in running speed during the prediscrimination phase of PSA. This would suggest that the decrease in speed was because of a general lethargic effect resulting from the relatively high dose of MK-801. The supposition that the discrimination impairment might have been a consequence of the altered prediscrimination phase resulting from a dose-dependent lethargy seems unlikely, being that rats treated with the less active isomer of MK-801 also showed impaired discrimination. However, this possibility needs to be considered and might explain why a learning impairment was not observed at a later age. In other words, a learning impairment might not have been observed in the adults because they recovered from the hypomotility effects of neonatal treatment.

Experiment 3 did not show a long-lasting impairment in nonspatial memory after neonatal treatment with MK-801. In spite of this, the likelihood of persistence of a nonspatial learning impairment is still questionable. There remains the possibility that the PSA task was not sensitive enough to show a learning effect in the older rats. PSA appears to be a relatively difficult task at weanling age, making it hard to assert that there was recovery of function from the impaired discrimination learning that was observed at weanling age. Amsel (22) suggests a relationship between the development of the hippocampus and the ontogeny of reward schedule effects. The argument can be made that paradoxical effects, such as the partial-reinforcement-extinction effect, require more hippocampal integrity than nonparadoxical effects, such as PSA. Indeed, although nonparadoxical learning can be impaired in young adult rats, the deficit in magnitude of PSA discrimination is not as strong as it is in preweanling (15) and weanling

rats. This might suggest that the long-lasting deleterious effects of neonatal NMDA blockade may be more robust in a spatial task (13) than on learning that is nonspatial in nature, but may not apply to nonspatial learning that is paradoxical in nature.

This work was supported by National Institute of Alcohol Abuse and Alcoholism Grant AA07052.

1. Balazs, R., Hack, N. & Jorgensen, O. S. (1988) *Neurosci. Lett.* **87,** 80–86.
2. Komuro, H. & Rakic, P. (1993) *Science* **260,** 95–97.
3. Sheetz, A. J. & Constantine-Paton, M. (1994) *FASEB J.* **8,** 745–752.
4. Gorter, J. A., Botterblom, M. H., Feenstra, M. G. & Boer, G. L. (1992) *Neurosci. Lett.* **137,** 97–100.
5. Gould, E. & Cameron, H. A. (1997) *Behav. Neurosci.* **111,** 49–56.
6. Sircar, R., Follesa, P. & Ticku, M. K. (1996) *Mol. Brain Res.* **40,** 214–220.
7. Butelman, E. R. (1989) *Pharmacol. Biochem. Behav.* **34,** 13–16.
8. Caramanos, Z. & Shapiro, M. L. (1994) *Behav. Neurosci.* **108,** 30–43.
9. Highfield, D., Nixon, K. & Amsel, A. (1996) *Behav. Neurosci.* **110,** 1–5.
10. Griesbach, G., Hu, D. & Amsel, A. (1998) *Behav. Brain Res.*, in press.
11. Fachinetti, F., Ciani, E., Dali'Olio, R., Virgili, M., Contestabile, A. & Fonum, F. (1993) *Dev. Brain Res.* **74,** 219–224.
12. Gorter, J. A., Veerman, M. & Mirmiran, M. (1992) *Brain Res.* **572,** 176–181.
13. Gorter, J. A. & de Bruin, J. R. (1992) *Brain Res.* **580,** 12–17.
14. West, J. R., Hamre, K. M. & Cassell, M. D. (1986) *Alcohol. Clin. Exp. Res.* **10,** 190–197.
15. Diaz-Granados, J. L., Greene, P. L. & Amsel, A. (1992) *Behav. Neurosci.* **106,** 940–946.
16. Lobaugh, N. J., Greene, P. L., Grant, M., Nick, T. & Amsel, A. (1989) *Behav. Neurosci.* **103,** 1159–1167.
17. Monyer, H., Burnashev, N., Laurie, D. J., Sakmann, B. & Seeburg, P. H. (1994) *Neuron* **12,** 529–540.
18. Sadile, A. G., De Luca, B. & Cioffi, L. A. (1978) *Abh. Akad. Wiss. DDR* **5,** 203–218.
19. Jacobson, W. & Cottrell, G. A. (1993) *J. Neurosci. Methods* **46,** 17–27.
20. Diaz-Granados, J. L., Greene, P. L. & Amsel, A. (1994) *Behav. Neural Biol.* **61,** 251–259.
21. Wigal, T. & Amsel, A. (1990) *Behav. Neurosci.* **104,** 116–126.
22. Amsel, A. (1992) *Frustration Theory: An Analysis of Dispositional Learning and Memory* (Cambridge Univ. Press, Cambridge, U.K.).