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RAPID COMMUNICATION

Inhibition of Nitric Oxide (NO) Production Selectively Impairs Learning and Memory in the Rat

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ESTALL, L. B., S. J. GRANT AND G. A. CICALA. *Inhibition of nitric oxide (NO) production selectively impairs learning and memory in the rat.* PHARMACOL BIOCHEM BEHAV **46**(4) 959-962, 1993.—Animals administered the nitric oxide (NO) synthase inhibitor N-*w*-nitro-L-arginine methyl ester (NAME) for five days exhibited severe deficits in acquisition of a place-navigation learning task. The effect of NAME was selective to place-navigation learning. NAME had no effect on sensorimotor or motivational processes in a related task. These results are consistent with the view that NO participates in learning and execution of memory tasks.

Nitric oxide N-*w*-nitro-L-arginine methyl ester *N*-methyl-D-aspartate (NMDA) Learning and memory Rat Behavior

RECENT evidence suggests that nitric oxide (NO) may act as a novel intracellular messenger in the central nervous system (14). In particular, NO has been implicated in long-term potentation (LTP) (12), a cellular model of learning and memory (3). It has been suggested that *N*-methyl-D-aspartate (NMDA) modulation of LTP may involve activation of NO synthase activity and NO production (12). For example, it has been demonstrated that NO synthase activity and NO are necessary for the production of LTP (12), and that inhibitors of nitric oxide synthase can block LTP (1,11,12). However, the relevance of these in vitro effects to learning and memory processes are not clear. Therefore, this study investigated the effects of N-*w*-nitro-L-arginine methyl-ester (NAME), an inhibitor of NO synthase, on a visible platform task and place-navigation learning in rats using a modified Morris swim task (9).

The place-navigation task used by Morris (9) was chosen because it is a spatial learning task which is solved on the basis of extra-maze cues. As with LTP, performance on this task is disrupted by administration of NMDA receptor antagonists (10). The effects of NAME on performance under these conditions were compared to the easier task of locating a clearly visible platform (10). Like Morris (10), we used the visible platform task, which does not require spatial learning but provides the same motivation to escape from the water, to distinguish between any secondary effects of NAME on motivation and sensorimotor performance and spatial learning per se.

METHODS

The experiment used 58 male Wistar rats approximately three months old (250-350 g). Animals were housed in groups of six to seven with free access to food and water and were maintained on a 12/12-h light/dark cycle.

Forty rats were randomly assigned to four equal groups (N = 10) and administered IP NAME (Sigma, St. Louis) (5.0, 10.0, or 20.0 mg/kg) or saline (volume 1.0 ml/kg, interdrug interval 30 min) twice a day for five consecutive training days. On each training day the animals had four training trials with a visible platform and extra-maze cues masked (visual discrimination task) (10) and five training trials 2 h later with a submerged invisible platform with clearly visible extra-maze cues (place-navigation task). This procedure was used because we wished to distinguish between a specific effect on spatial learning as opposed to effects of NAME on sensorimotor or moti-

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vational processes. The visible platform task provided the same motivation to escape from the water and required the same sensory motor skills as the place-navigation task but did not require spatial learning.

To test for cumulative drug effects due to the order of testing, place-navigation training preceded the visible platform task in a second group of animals (saline, $N = 9$; NAME 20.0 mg/kg, $N = 9$). No other procedural changes were introduced.

All training was conducted in a pool constructed of wood (7 ft diameter, 2 ft deep) and lined with a blue vinyl pool liner filled to a depth of 20 cm with opaque water at room temperature (20–22°C).

In the visible platform task the animals were placed singly into the water facing the wall at randomly determined start positions. They were allowed 40 s (per trial) to locate and climb onto the visible platform. Animals had to stay on the platform for 10 s before removal (if they fell off the platform they were placed back on the platform until 10 s had elapsed). Rats that failed to climb onto the platform within the allotted time were placed on the platform for 10 s and then removed until the next trial. The visible platform was a black-and-white-striped Plexiglas box (22 cm high and 14 × 12 cm wide) placed in the pool with its top surface 2 cm above water level. Black PVC liner surrounded the perimeter of the pool masking all extra maze cues. On each day the visible platform was moved to a new location to prevent the adoption of a spatial learning strategy.

During place-navigation training the extra-maze cues were made visible so that the rats could learn the location of the submerged platform (19 cm high and 14 × 12 cm wide) on the basis of these cues. On each trial start positions were randomly determined and the animals were allowed 90 s to complete the trial. The rats' task was to locate the submerged platform (which was placed in a fixed position in the centre of a circular maze, 64 cm from the wall and 1 cm below the water surface) and climb onto it. They were required to remain on the platform for 20 s (if they fell off the platform they were placed back on the platform until 20 s had elapsed) before they were removed and placed in a holding cage (intertrial interval 10 s). Those that failed to find the platform were placed onto the platform for 20 s and then removed until the next training trial.

The data for the visible platform and place-navigation training trials were analysed separately within each order of testing component. On each day the data from each component of the experiment were collapsed across trials to obtain the mean latency for each animal. As the repeated-measures analysis of variance (ANOVA) indicated nonsignificant Drug × Days interaction in all components of the experiment the scores for each individual day were subjected to a one-way independent ANOVA. Individual comparisions were made between NAME and saline by using the Dunnett's t test.

RESULTS

Trial latencies for the visible platform task over the five days of training are presented in Fig. 1. Performance improved with training but no significant effects of NAME were obtained—day 1: $F(3, 36) = 2.44$, p = n.s.; day 2: $F(3, 36) = 2.31$, p = n.s.; day 3: $F(3, 36) = 1.43$, p = n.s.; day 4: $F(3, 36) = 1.56$, p = n.s.; day 5: $F(3, 36) = 1.78$, p = n.s. In sharp contrast, NAME produced a dose-dependent impairment on the first two training days in the place-navigation task—training day 1: $F(3, 36) = 7.57$, $p < 0.005$; day 2: $F(3, 36) = 3.11$, $p < 0.03$ (Fig. 2). On day 1 of place-navigation training NAME produced a severe impairment at 10.0 mg/kg ($p < 0.05$, Dunnett's t test) or 20.0 mg/kg ($p < 0.01$, Dunnett's t test), but not at 5.0 mg/kg. By day 2 of place-navigation training only animals administered the highest dose of NAME (20.0 mg/kg) exhibited impaired learning ($p < 0.05$, Dunnett's t test).

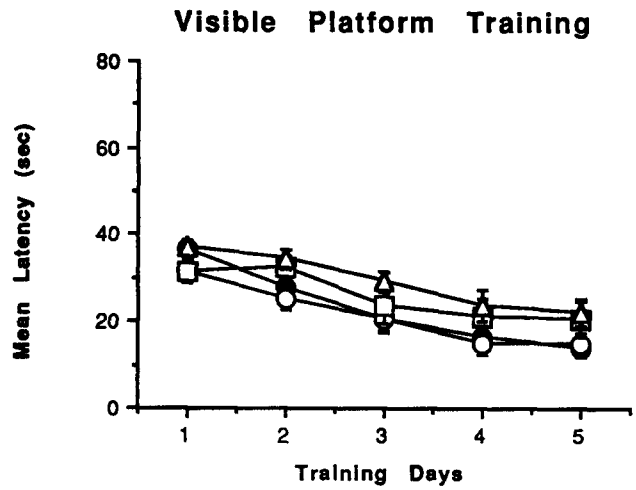

FIG. 1. Mean latency of NAME- and saline-treated animals in locating the visible platform on each training day when tested first on the visible platform task. There were no differences between the groups. ● saline; ○ NAME 5.0 mg/kg; □ NAME 10.0 mg/kg; △ NAME 20.0 mg/kg.

Reversing the order of training did not alter the overall pattern of results. In fact, animals given place-navigation training prior to visible platform training were somewhat more impaired in both tasks (Figs. 3 and 4). NAME (20 mg/kg) retarded place-navigation learning for a longer period of

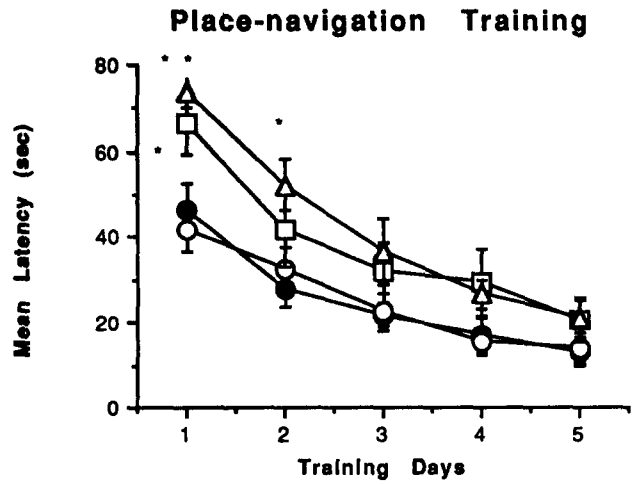

FIG. 2. Mean latency of NAME- and saline-treated animals in locating the submerged platform on each training day when tested first on the visible platform task. NAME-treated animals were significantly slower on day 1 (*$p < 0.05$; **$p < 0.01$, Dunnett's t test) and day 2 (*$p < 0.05$, Dunnett's t test) of training. ● saline; ○ NAME 5.0 mg/kg; □ NAME 10.0 mg/kg; △ NAME 20.0 mg/kg.

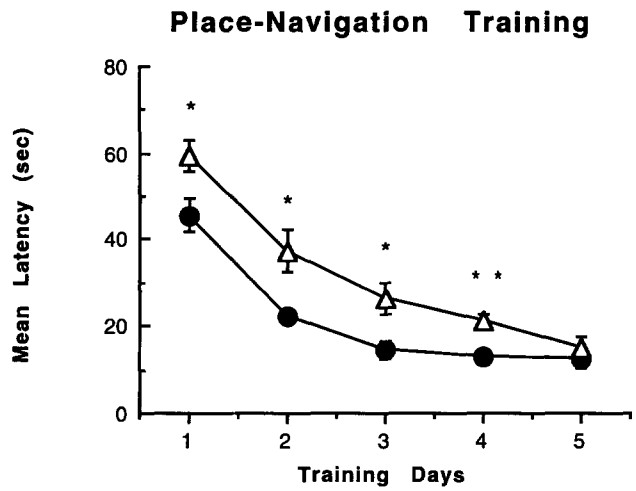

FIG. 3. Mean latency of NAME- and saline-treated animals in locating the submerged platform on each training day when tested first on the place-navigation task. NAME-treated animals were significantly slower on days 1-3 (*$p < 0.05$, Dunnett's t test) and day 4 (**$p < 0.01$, Dunnett's t test) of training. ● saline; △ NAME 20.0 mg/kg.

time (Fig. 3). Significant reductions in performance were seen for days 1-4—day 1: $F(1, 16) = 6.32$, $p < 0.02$; day 2: $F(1, 16) = 8.24$, $p < 0.01$; day 3: $F(1, 16) = 7.60$, $p < 0.01$; day 4: $F(1, 16) = 10.41$, $p < 0.005$—and with Dunnett's t test, days 1-3, $p < 0.05$; day 4, $p < 0.01$. Again, NAME did not affect performance in the visible platform task on days 1-4—day 1: $F(1, 16) = 3.98$, p = n.s.; day 2: $F(1, 16) = 1.14$, p = n.s.; day 3: $F(1, 16) = 2.50$, p = n.s.; day 4: $F(1, 16) = 1.36$, p = n.s.—except for a small but significant impairment on the final training day, $F(1, 16) = 4.80$, $p < 0.04$ (Fig. 4).

Taken together, the data show that all groups eventually

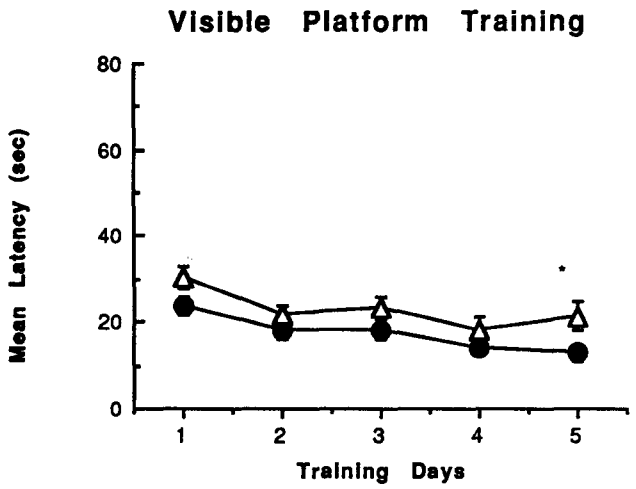

FIG. 4. Mean latency of NAME- and saline-treated animals in locating the visible platform on each training day when tested first on the place-navigation task. NAME-treated animals were significantly slower on day 5 (*$p < 0.05$, Dunnett's t test) of training. ● saline; △ NAME 20.0 mg/kg.

mastered place-navigation learning. Animals given NAME exhibit a dose-response impairment in the acquisition of place-navigation learning. NAME causes a selective impairment of place-navigation learning and does not affect learning in the visible platform task. Ordering of training tasks cannot account for these results.

DISCUSSION

There are several conclusions which can be drawn from this experiment. The most important of these is that NAME, a NO synthase inhibitor, produced a dose-dependent impairment in the acquisition of spatial learning. Given proposals that NMDA receptors initiate NO synthesis (5,6,13), it is significant to note that the effects of NAME in this study are similar to those reported for the *N*-methyl-D-aspartate receptor antagonist aminophosphonovaleric acid (AP5) in a similar visual discrimination and place-navigation learning task (10). $_{D,L}$-AP5 caused a selective impairment of place-navigation learning without affecting visual discrimination learning. Moreover, the effects of NAME are similar to those reported for MK-801 in the place-navigation task (8). MK-801 slowed learning when administered before training, but not after. These results lend credence to hypotheses linking NMDA receptor activation to induction of NO synthesis during learning. As experiments have shown that inhibitors of nitric oxide synthase can block LTP (1,11,12), and since it has been indicated that $_{D,L}$-AP5 blocks the induction of hippocampal LTP (2,7), which is hypothesised to be important in spatial learning, the present results suggest that NAME may be producing its learning impairment by a similar neural mechanism.

It is likely that the treatment regime used in this study produced a substantial reduction of NO synthesis. Previously it has been shown that a single injection of NO_2ARG produced 50% inhibition and four days of administration produced 95% inhibition of NO synthase, but there was no further inhibition thereafter (4). The effects of NAME on learning in this study had a similar time-course in that there were no further deficits in learning after four days of training.

This research also shows that NAME in the doses tested retards but does not prevent the eventual learning of the place-navigation task. Over the five training days performance progressively improved to the point where NAME-treated rats performed the task as well as their vehicle controls. Similar results have been reported for $_{D,L}$-AP5 (10), low but not high doses of MK-801 (8), and a higher dose of NAME (40.0 mg/kg, data not presented); however, we do not know if this holds for other drug regimes.

These results also provide clear evidence that NAME as administered in the present study does not impair learning in the visible platform task. When extra-maze cues were eliminated and the platform was made visible, presumely making the task less difficult than that for place-navigation learning, NAME did not retard learning under these circumstances.

The selective effect of NAME on the two learning tasks is not due to factors responsible to order effects. NAME retarded place-navigation learning and not learning in the visible platform task regardless of which task preceded the other on each of the five training days. However, it is difficult to ignore the difference in latency scores for place-navigation training under 20 mg/kg NAME (Figs. 2 and 3). The group which received visible platform training first is clearly inferior to the group that received place-navigation first, on all training days. In the former group, performance may have been more impaired from having received visible platform training 2 h prior

to place-navigation training, which might have caused the rats to waste time looking for the visible platform. Rats that had received visible platform training 22 h prior to place-navigation training may not have been as inclined to remember and therefore seek out the visible platform.

The selective effects of NAME on the two tasks suggests that NAME does not seem to produce gross sensory or perceptual deficits. On visible platform training the rats seem to see and organize the platform cues provided they are part of the platform. The speed with which NAME-treated rats execute the response when the platform is visible also suggests that their motive to leave the water is unaffected. The difference between the tasks is that when the rats are given place-navigation training they are required to infer the platform location from available extra-maze cues. The pattern of results from the present study suggests that NAME interferes with the ability of rats to image the platform location or the association of extra-maze cues with these images. Identification of the neural mechanisms underlying these effects awaits future research.

In conclusion, the results show that 1) NAME impairs place-navigation learning but has no effect on the visible platform task, indicating that NAME has no effect on sensory processes, performance, or motivational factors, and 2) although the learning impairment is relatively severe, the learning deficits can be overcome. These results provide behavioral evidence that attenuation in NO synthase activity can influence the acquisition process.

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