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The Effect of NMDA Antagonists in the Radial Arm Maze Task With an Interposed Delay

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BUTELMAN, E. R. *The effect of NMDA antagonists in the radial arm maze task with an interposed delay*. PHARMACOL BIOCHEM BEHAV **35**(3) 533–536, 1990.—N-Methyl-d-aspartate (NMDA) receptors mediate the triggering of hippocampal long-term potentiation (LTP), a current physiological model of memory. This model was tested in the rat through the effect of (+)-5-methyl-10,11-dihydro-5H-dibenzo [a,d] cyclohepten-5,10-imine maleate (MK-801, a novel noncompetitive NMDA antagonist) on the radial arm maze (RAM) task with a 15-minute delay interposed at the midpoint choice. In two separate experiments, substereotypical drug doses of MK-801 and phencyclidine (a dissociative anesthetic with NMDA antagonist properties) were given intraperitoneally, before the trial or at the start of the delay. "Efficiency" was impaired in both tasks, but near-instantaneous use of encoded information seemed to be unaffected. This evidence would support a proposed role for NMDA-mediated pathways (and possibly LTP) in delayed stages of memory formation or use.

NMDA receptor MK-801 Phencyclidine Long-term potentiation Radial arm maze Working memory

LONG-TERM potentiation (LTP) is a widely studied physiological model of memory (13). It consists of a sustained increase in synaptic efficiency following short bursts of afferent tetanic stimulation, and has been observed mainly in the hippocampus (3). An excitatory amino acid receptor subtype, the NMDA receptor, mediates the triggering but not the maintenance of LTP and is also widely present in the hippocampus (3). Thus, it was predicted that MK-801 (a selective NMDA antagonist which can exert its effect when peripherally administered) would cause learning and memory deficits (2). The prediction has been supported by recent evidence in the rat, showing that MK-801 treatment results in deficits in continuous nonmatching to sample (10), olfactory discrimination learning (12), reversal training in the T-maze (14) and spatial navigation (11) as well as in the RAM task (manuscript in preparation).

It has been proposed that treatments which interfere with LTP (including the administration of NMDA antagonists) would result in impairments of stimulus encoding (6).

"Working memory" tasks [such as the radial arm maze (RAM) task] would be especially sensitive to NMDA antagonist treatment, due to their dependence on hippocampal function (8). Milner (7) proposed a reworking of the Hebbian theory of "cell assembly" as a basis for hippocampal amnesia. He proposed a two-step encoding process in which recently sampled stimuli are held in a "reverberatory" cell assembly (4) before being coded by longer-lasting synaptic changes (such as LTP).

The treatments studied were intraperitoneal doses of MK-801 and phencyclidine [PCP, a drug of abuse with potent NMDA antagonist properties, which also causes impairments in the RAM, see (5)] at doses which would not affect locomotor activity or overt behaviour.

The aim of the present experiments was to further study the effect of NMDA antagonist treatment in the RAM paradigm, by the adoption of a 15-minute delay between the fourth and fifth arm entries within a trial (1), with drug administration occurring at the onset of the interposed delay (Experiment A) or before the start of a trial (Experiment B). This version of the RAM task allows the categorisation of errors as being caused primarily through retrieval or encoding impairments, which is of particular interest in view of the proposed role of LTP in memory processes.

METHOD

Subjects

Subjects (Ss) were 12 and 14 male Lister Hooded rats (Harlan Olac, Bicester, Oxon, UK) for Experiments A and B, respectively, with weights at the start of the experiments of 200–220 g. They were gradually food deprived to approximately 85% of their expected free-feeding weight, by feeding 30 minutes after the end

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TABLE 1

EFFICIENCY AND RATE OF ARM ENTRY IN POSTDELAY STAGE OF EXPERIMENT A

Treatment (mg/kg)	Mean Efficiency	Mean Time per Arm Entry (sec)
Saline	83 (5.2)	16.9 (1.1)
0.1 MK-801	81 (4.7)	16.8 (1.0)
0.2 MK-801	68 (4.6)	16.3 (2.6)
4 PCP	70 (4.7)	17.1 (2.0)

See definition of efficiency in text. SEM in parentheses.

of each behavioural session. All behavioural sessions took place between 0900 and 1300.

Apparatus

An 8-arm radial maze, made wholly of unpainted aluminum (9), was used. The central platform was octagonal in shape (side 14 cm): the arms were 75 cm long and 7.5 cm wide (slightly tapering at the end proximal to the central platform), with 0.5 cm high side walls.

The maze was elevated 50 cm from the floor in a dimly lit room with numerous visual extra-maze cues. A small circular food well was placed at the distal end of each arm; one Noyes (45 mg) pellet was placed in each well during training and testing. A circular start box was used, in which the S was placed before the start of a trial (and after the interposed delay) on the central platform. The box was lifted to allow the S to enter the arms. The Ss were kept in a separate large cage during the interposed delay.

Design

A simple repeated measures design was used in each experiment, with 4 conditions: saline control, 0.1 and 0.2 mg/kg MK-801 and 4 mg/kg PCP (substituted by 3 mg/kg PCP in Experiment B, to avoid occasional stereotypical effects observed at the higher dose). Ss underwent training in the RAM task with a 15-minute delay interposed between the fourth and fifth arm entry until they reached criterion. They were then tested under the four conditions in balanced order (test days were separated by at least 48 hours and one training trial at criterion level).

Procedure

Ss were handled daily for five days prior to the start of the studies. They were initially shaped until they ate a pellet placed in each food well. Training was then started (1 trial/day, 5–7 days/week) in the standard RAM task with one pellet per arm.

In RAM training, the S was placed in the start box and after 5 seconds the box was lifted to signal the start of the trial, which ended when all the arms had been entered or after 5 minutes. Arm entries were scored visually as the presence of four paws within one arm. After 12 RAM training trials (24 trials in Experiment B), training in the delayed version of the task was begun. The S was removed from the maze after the fourth arm entry and placed in the delay cage for 15 minutes, after which it was returned to the start box of the maze for the postdelay stage; only the four previously unentered arms were baited at this stage.

In Experiment A, Ss were injected with the appropriate solution IP 2 minutes after the onset of the delay, while in Experiment B they were injected 15 minutes prior to the start of the trial.

TABLE 2

ERROR TYPE IN POSTDELAY STAGE OF EXPERIMENT A

Treatment (mg/kg)	Mean Number Pre Errors (SEM)	Mean Number Post Errors (SEM)
Saline	2 (0.6)	0.1 (0.1)
0.1 MK-801	2.3 (0.6)	0.1 (0.1)
0.2 MK-801	3.5 (0.8)	1.2 (0.7)
4 PCP	3.3 (1.0)	1.1 (0.5)

See text for definition of Pre/Post errors.

Performance in test trials was measured by the number of errors and by "efficiency" values (5), where efficiency = 8/total number of arms to completion, expressed as a percentage. An efficiency value of 100, therefore, represents errorless performance.

Drugs

The compounds used were MK-801 (Merck Sharp and Dohme, Harlow, UK) and phencyclidine hydrochloride (Sigma, UK). Fresh drug solutions in saline were prepared on each test day. Control Ss received vehicle solutions of the appropriate volume.

RESULTS

Efficiency deficits were observed in Experiments A and B. However, the pattern of errors caused in the two experiments was not identical. Activity levels (as monitored by the time/arm entry) were not affected. The Ss' overt behaviour was likewise unaffected and they still followed the "reference memory" procedure required by the task (i.e., entering different arms in search of food rewards). Slight stereotypical effects were occasionally observed in the PCP conditions; when these occurred the trial was suspended and rerun after an interval not shorter than 48 hours and a trial at the criterion level of performance. The 0.05 level of significance was adopted throughout.

Experiment A

All Ss reached the criterion level of performance. The mean number of trials to criterion was 32 (SEM 2.8, range 19 to 50).

Activity levels (as monitored by the rate of arm entry) were not affected by treatment (see Table 1).

Efficiency was impaired by treatment. A significant treatment effect was observed, $F(3,33) = 3.4$, while an analysis of the saline and MK-801 doses alone yielded a significant linear trend, $F(1,11) = 4.97$ (see Table 1). A Dunnett's test revealed that efficiency levels in the 0.2 mg/kg MK-801 condition were significantly reduced with respect to control.

As expected, all errors occurred during the postdelay period. Interestingly, it was found that the majority of errors during the postdelay period were made as reentries to arms visited originally in the predelay period ("Pre" errors) rather than to arms originally visited earlier in the postdelay stage ("Post" errors) (see Table 2). Thus MK-801 and PCP caused deficits in the use of very recently encoded information, but only if the stimulus had been experienced several minutes earlier (hence the reentries to arms visited before the delay). However, it has to be noted that the chance probability of a Pre error is higher than that of a Post error throughout the task. Furthermore, recency effects would be expected to result in more reentries to arms visited at a temporally more remote stage.

Experiment B

All but 2 Ss achieved the criterion level of efficiency within 60

TABLE 3

EFFICIENCY AND RATE OF ARM ENTRY IN EXPERIMENT B

Treatment (mg/kg)	Efficiency (SEM)	Predelay Stage (SEM)	Postdelay Stage (SEM)
Saline	87.1 (3.2)	14.3 (1.6)	14 (1.7)
0.1 MK-801	78.7 (4.9)	12 (0.9)	12.9 (1.7)
0.2 MK-801	65.1 (5.4)	14.9 (1.3)	14.5 (1.1)
3 PCP	74 (4.7)	14.9 (1.6)	13.4 (1.4)

Mean time/arm entry (sec) during predelay and postdelay stages. See text for definition of efficiency.

TABLE 4

ERROR TYPE IN POSTDELAY STAGE OF EXPERIMENT B

Treatment (mg/kg)	Mean Number Pre Errors (SEM)	Mean Number Post Errors (SEM)
Saline	1.3 (0.3)	0.1 (0.1)
0.1 MK-801	2.2 (0.7)	0.6 (0.2)
0.2 MK-801	4.4 (0.9)	1.0 (0.3)
3 PCP	2.8 (0.6)	0.6 (0.2)

See text for definition for Pre/Post errors.

trials. The mean number of trials to criterion was 28 (SEM 3.8, range 12 to 53).

The overall level of activity was not affected by treatment as shown by the lack of significant effects in a 2×4 ANOVA (stage of the trial $\times$ treatment) on time/arm entry for the predelay and postdelay stage (see Table 3).

An ANOVA on efficiency levels yielded a significant treatment effect, $F(3,39) = 4.39$. Furthermore, an analysis with saline and MK-801 doses yielded a significant linear trend, $F(1,13) = 10.21$. A Newman-Keuls test confirmed that efficiency in the 0.2 mg/kg MK-801 condition was lower than in the saline condition. Thus, MK-801 dose dependently caused deficits in efficiency (see Table 3).

Only 1 error in the whole experiment occurred in the predelay stage (in the 3 mg/kg PCP condition), thus confirming the finding from Experiment A that MK-801 does not cause deficits in the use of very recently encoded information.

Errors occurring during the postdelay period were classified in Pre or Post types as in the previous experiment. Separate analyses were carried out on the number of errors of each type. Both were significantly increased by treatment: Pre: $F(3,39) = 4.16$, Post: $F(3,39) = 3.42$. Linear trends were also found for saline and MK-801 doses: Pre: $F(1,13) = 8.22$, Post: $F(1,13) = 9.27$. Thus, both types of errors were increased by MK-801 (see Table 4).

DISCUSSION

Experiment A

Efficiency in the task was impaired by MK-801 and PCP. Most errors were reentries in the postdelay stage to arms entered before the 15-minute interval. The low level of Post errors caused by the treatment would indicate that near-instantaneous use of information was not affected. It would appear, therefore, that only the later stages of the encoding process are impaired by NMDA antagonists. This would support the proposal that "long-term synaptic changes" (possibly exemplified by LTP) subserve the second, delayed part of the encoding process (7). However, a possible effect on the recall of information after delays in the order of minutes may not be discounted.

Experiment B

Overall efficiency was also dose-dependently impaired by MK-801 and PCP in this task. The finding that errors were not increased by treatment in the predelay stage (which was under the effect of MK-801 or PCP), while Pre errors were significantly increased, confirms the finding from the previous experiment that MK-801 causes deficits when memory for stimuli needs to span several minutes at least. The lack of errors during the predelay stage would suggest that nonspecific treatment effects (e.g., on motor function or response strategies) did not occur. The increase in Post errors in this experiment suggests that errors after short delays may, however, occur when the load on memory is increased (such as could be caused by the large number of arm entries occurring during the postdelay stage).

General

The hypothesis that NMDA antagonists would cause deficits in a delayed stage of the encoding process was partially supported by the data. The present findings, however, do not allow the exclusion of a possible recall deficit occurring when delays are several minutes long. The issue could be resolved by more parametric studies on the effect of NMDA antagonists in this paradigm, centering on variations in the length of the interposed delay.

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