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BRIEF COMMUNICATION

Phencyclidine Impairs Temporal Order Memory for Spatial Locations in Rats

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LONG, J. M. AND R. P. KESNER. *Phencyclidine impairs temporal order memory for spatial locations in rats.* PHARMACOL BIOCHEM BEHAV 52(3) 645–648, 1995.—The effects of phencyclidine (PCP), an NMDA antagonist, was assessed on a complex task that has been shown to be dependent on hippocampal function. This task required memory for the temporal order of spatial locations. Rats were given IP injections of saline or PCP (3–4 mg/kg) on a double alternation schedule. With PCP injections rats were severely impaired relative to saline injections. Furthermore, PCP was shown to have no effects on the ability of rats to discriminate three-dimensional objects (a task that is not dependent on hippocampal function). The present data, in conjunction with previous results, suggest that the involvement of NMDA receptors in the hippocampus might be a function of the complexity of the task.

Phencyclidine Hippocampus NMDA Rats Learning and memory

THE NMDA subtype of the glutamate receptor is thought to be involved in mediating specific types of learning; specifically, learning that is mediated by the hippocampal formation (15). The NMDA receptor is also involved in the induction of long-term potentiation (LTP) (13), a process that has been postulated as a model of memory formation. Although located throughout the CNS, the highest density of NMDA receptors is in the hippocampal formation, especially in the CA1 subfield. Phencyclidine (PCP), an NMDA antagonist, blocks LTP in the CA1 region and dentate gyrus of the hippocampal formation (1,3,16) and produces deficits in tasks that are mediated by the hippocampal formation (5,12,14), thus suggesting that NMDA receptors in the hippocampal formation are involved in tasks that involve the entire hippocampus.

However, in experiments that assessed working memory for a single spatial location on a radial arm maze, hippocampal lesions produced marked deficits, whereas the NMDA antagonist PCP did not (7,9). In this task, rats were placed on an eight-arm radial maze and allowed to visit a randomly selected arm for food reward. After returning to the center, the rat was presented with two arms, the arm just visited, and a new arm. To receive further food reward, the rat had to select the arm that was previously visited. Rats with hippocampal lesions performed at chance in this task, whereas rats administered 4 mg/kg PCP showed no impairment. These data suggested that there are some tasks that are mediated by the hippocampus, but not necessarily by the NMDA subtype of the glutamate receptor. More specifically, working memory for a single spatial location does not seem to involve the NMDA receptor.

In a task that required continuous recognition memory for multiple spatial locations, lesions of the hippocampal formation resulted in a robust impairment (6), whereas administration of PCP resulted in only a mild impairment (8). In this task, rats had to remember which arms were part of a list of spatial locations that had been previously presented that day. These data support the hypothesis that NMDA receptors become at least partially recruited as the demands of the task become more complex. The continuous recognition task differs from the working memory for a single location by increasing the number of spatial locations to be remembered, thus increasing the mnemonic demand.

Hippocampal lesions result in complete deficits in a task that assesses memory for temporal order information for spatial locations (2,10). In this task, rats had to remember the temporal order of a list of spatial locations that had been

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presented that day, thus adding an additional temporal dimension not involved in the previous task (i.e., the recognition task).

The purpose of Experiment 1 was to determine whether the dimension of temporal order would recruit an increased number of NMDA receptors in the hippocampal formation. If this occurs, then deficits for NMDA receptor blockade by PCP should be similar to deficits seen after lesions of the hippocampal formation. The purpose of Experiment 2 was to serve as a control for possible sensory-motor, motivation, and nonhippocampal memory dysfunction. It was thought important to test the rats in a task that is not hippocampal dependent, an object discrimination task (15). If PCP injections resulted in performance deficits on the hippocampal dependent task in Experiment 1, but not in the nonhippocampal-dependent task in Experiment 2, then this would suggest that action of PCP could be on the NMDA receptors of the hippocampus.

METHOD

Experiment 1: Memory for Temporal Order of Spatial Location

Subjects. Subjects were eight male hooded rats weighing between 350 and 420 g, housed individually in a colony maintained on a 12 L : 12 D cycle. All rats had free access to water, with food restricted to maintain each rat at approximately 85-90% of its free-feeding weight. All testing was conducted during the light portion of the light : dark cycle.

Apparatus. All testing was conducted on a single eight-arm radial maze. Briefly, the maze consisted of a center platform with a diameter of 42 cm, and eight arms projecting from the center platform. Both the center platform, and the arms, were constructed of wood, painted white, standing 100 cm above the floor. The arms were 71 cm long × 9.5 cm wide, and had Plexiglas walls 5.7 cm in height. A food well, 2.5 cm in diameter, was drilled 1.5 cm deep at the distal end of each arm. A series of Plexiglas guillotine doors were located at the base of each arm, allowing the experimenter to dictate the availability of any arm; the doors were operated manually via a pully system. In the gaps between the doors on the central platform, strips of Plexiglas were installed to prevent the rats from gaining access to the arms by climbing between the doors. When all doors were closed, this had the effect of forming a continuous ring around the center platform. A poster board cylinder 32 × 122 cm could be placed around the rat in the central platform, thus blocking the rat's view of the room. The room in which the maze was located was well lit and had six large pictures on the wall to help serve as spatial markers. The room had no windows and one door. Froot Loops (Kellogg's) cereal served as the food reward.

Familiarization pretraining. Each rat was acclimated to the maze before temporal order testing procedures began. For the first several days the rat was placed on the center platform with all of the maze doors open. Food, approximately 20-1/2 pieces, was placed throughout the maze. These pretraining trials lasted 10 min or until the rat had eaten all of the food. Temporal order testing began when rats readily visited arms upon opening of a door.

Temporal order training. Each rat received one trial per day, with each trial consisting of a study phase and a test phase. In the study phase, the rat was placed on the center platform with all doors closed. The rat then visited each arm in a pseudorandomized forced choice sequence. Food was available in the well at the end of each arm. The sequence was presented to the rat by opening only one door at a time, and keeping all other doors closed. In this way, the rat could only visit the arms in the order dictated by the experimenter. Immediately after the rat returned to the center platform from the last arm presented in the sequence, the test phase began. The cylinder was placed around the rat (thereby blocking its view of the arms and the experimenter), and one of the arms was rebaited. Two doors were then opened, the cylinder removed, and the rat was allowed to visit an arm. To obtain further food reinforcement, the rat had to visit the arm that occurred earlier in the sequence for that day. Only one test was given for each trial, and temporal lags of 0, 2, 4, or 6 were pseudorandomly selected for each test phase. Temporal lag refers to the number of intervening arms that occur between the two test arms opened in the study phase. For example, a temporal lag of 0 refers to the case when the two arms selected in the test phase were temporally adjacent in the study phase. A temporal lag of 6, refers to the case when the two arms selected in the test phase were separated in the sequence by visits to six other arms. Rats were trained until they reached a criterion of 75% correct for temporal lags of 2, 4, and 6, based on eight observations of each lag condition. Once this criterion was met, drug administration trials were begun.

Using a within subject design, all rats received injections (IP) of PCP (3 or 4 mg/kg) or saline 30 min prior to testing, in volumes equivalent to 0.1% body weight on a double alteration schedule. This meant that the rats received 2 days of PCP followed by 2 days of saline, until each rat had received eight sessions of saline and eight sessions of PCP for each lag. The location of the IP injections were spaced such that no injection area was used twice in the same week. Two of the rats received 3 mg/kg PCP, because the 4 mg/kg dose produced sensory-motor problems. The results were analyzed with an ANOVA (treatment × lag) and Newman-Keuls post hocs.

Experiment 2: Object Discrimination

This experiment was designed to assess the effects of PCP injections on object discrimination and to explore the possibility that the differences observed in Experiment 1 were due to visual/sensory motor factors rather than memory processes.

Subjects. Five of the rats in Experiment 1 served as subjects as well as five Long-Evans rats that had previous experiences in an unrelated task.

Apparatus. All testing was conducted on a cheese board maze, which has been described in detail previously (14). Briefly, the maze was circular, made of 3.3 cm thick wood, painted white, with a diameter of 119 cm. The maze was elevated 26 cm from the floor and contained 117 holes 2.4 cm in diameter and 1.1 cm deep. The holes were evenly spaced with an average distance of 2 cm between holes. The two objects to be discriminated were a yellow spongy ball and a green wooden triangle. The pretraining objects consisted of a wooden cube painted black and a wooden cylinder painted white. All objects were approximately the same size. Froot Loops cereal served as the food reward.

Pretraining procedure. Two holes approximately in the center of the cheese board served as food wells throughout pretraining. Pretraining lasted approximately 5 days. At first, food was stacked in the wells so that it was visible to the rat. The pretraining objects were placed behind the food well, and the rat was placed on the perimeter of the maze facing the objects. After the rat had learned to approach the objects and eat the reward, the objects were gradually placed over the wells, until the objects completely covered the food.

Testing procedure. All rats were pseudorandomly assigned to one of two treatment groups: four rats from Experiment 1 received 3-4 mg/kg PCP injections, one rat from Experiment 1 and five rats with unrelated experience received saline control injections. Thirty minutes prior to testing each rat was given an IP injection and returned to their home cage. All rats were given one test session per day, consisting of 20 trials. The same two spatial locations used in pretraining were used throughout the experiment. The test objects were placed over the two holes on each trial; the particular location on any given trial was randomized. For each rat only one of the objects served as the positive stimulus (i.e., had food reward underneath). To receive reinforcement, the rat had to displace only the positive stimulus. Displacement of the negative stimulus was recorded as an error. Rats were trained until they had reached a criterion of 80% correct for 3 consecutive days. The results were analyzed by a two-tailed Mann-Whitney *U*-test.

RESULTS

Experiment 1

The two rats that received 3 mg/kg PCP did not differ from the six rats that received 4 mg/kg PCP; thus, they were combined to form a single group. The effects of PCP or saline on mean percent correct performance for temporal order are shown as a function of lag in Fig. 1. The results show the temporal lag effects in both conditions as performance improved for all lags greater than zero. However, compared to the saline condition performance in the PCP condition was significantly impaired for lags 4 and 6. An ANOVA (2 × 4) revealed a significant main effect for treatment (PCP/saline), $F(1, 7) = 30.7$, $p < 0.01$, and for temporal lag, $F(3, 21) = 12.40$, $p < 0.01$, but no significant treatment by temporal lag interaction, $F(3, 21) = 2.43$, $p > 0.05$. Subsequent Newman-Keuls tests revealed that the temporal lag of 0 differed from the lags of 2, 4, and 6 ($p < 0.05$) for both conditions.

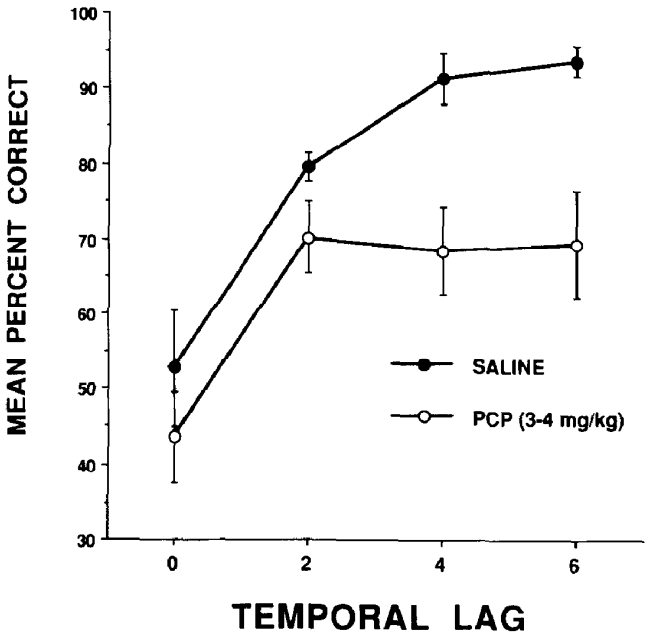

FIG. 1. Mean percent correct performance (±SEM) for saline (●) and PCP (3-4 mg/kg) (○) trials as a function of temporal lag.

The temporal lags of 2, 4, and 6 did not significantly differ among each other ($p > 0.05$). Further Newman-Keuls test revealed that for temporal lags of 0 and 2, PCP trials did not differ significantly from saline trials ($p > 0.05$), but for temporal lags of 4 and 6, PCP trials were significantly impaired compared to saline trials ($p < 0.05$). No sensorymotor impairments were seen at the 4 mg/kg doses for six of the rats; for two rats that experienced motor problems, a dose of 3 mg/kg was used for all PCP trials. The rats that received the 3 mg/kg dose of PCP showed impaired coordination at the 4 mg/kg dose and no increase in locomotor activity. This is in agreement with an earlier study in which rats receiving a 4 mg/kg dose of PCP did not differ from controls in locomotor activity (11).

Experiment 2

The saline control rats that had experience from Experiment 1 did not differ from the saline control rats that had unrelated experiences and, therefore, were combined to form a single group. The mean number of trials to criterion for the rats in the saline condition was 6.83 and rats in the PCP condition had a mean of 10.25. A two-tailed Mann-Whitney *U*-test revealed that there was not a significant difference between the groups ($U = 13.5$, $n = 4,6$, $p > 0.05$).

DISCUSSION

The results of Experiment 1 provide further support for the existence of the temporal lag effect in rats for memory of spatial order information. Furthermore, the results indicate that systemic administration of PCP, a competitive NMDA antagonist, results in impaired memory for spatial temporal order information compared to systemic administration of saline. As this task has been shown to be sensitive to hippocampal lesions (2) and the observation that there are a high density of NMDA receptors in the hippocampus, it is very likely that the site of action of the systemic injections was the hippocampal formation. Additional support for this hypothesis can be derived from the results of Experiment 2, a nonhippocampal mediated object discrimination task (15). In this experiment, rats that received systemic injections of PCP at doses identical to those that impaired memory for Experiment 1 were not impaired. So, while not discounting the possibility that the site of action of the systemic injections could include other brain regions, it is likely that the present results are due to NMDA receptor blockade in the hippocampal formation.

The deficits seen as a result of NMDA blockade by PCP in the memory for temporal order task are similar to those seen after lesions of the hippocampal formation, although not as robust. This is in contrast to the continuous recognition task where PCP resulted in small impairments compared to those seen after hippocampal lesions (6,8) and the working memory for a single location task in which PCP produced no deficits compared to complete deficits with hippocampal lesions (7,9). The data suggest that NMDA receptors in the hippocampus are not required to perform the working memory for a single location task, but when the number of spatial locations to be remembered becomes larger than one, as in the continuous recognition task, then NMDA receptors in the hippocampus are recruited. Furthermore, if the additional dimension of temporal order is added, as in Experiment 1, then even more NMDA receptors become involved. The observation that PCP blockade of NMDA receptors does not exactly mimic the deficits seen after hippocampal lesion suggests that there are other non-NMDA processes within the hippocampus that are in-

volved in the temporal order task, perhaps opiate receptor-mediated LTP (4), or that the present doses did not completly block hippocampal NMDA receptors. At the present doses of 3-4 mg/kg, there were no observed sensory-motor impairments, which is further supported by the observation that PCP did not impair object discrimination learning in Experiment 2.

In conclusion, the present results indicate that systemic administration of PCP, a competitive NMDA antagonist, results in impaired memory for spatial temporal order information compared to systemic administration of saline. These deficits are similar, but not identical, to deficits seen after lesions of the hippocampus. Furthermore, the results suggest that NMDA involvement in spatial tasks increases as the complexity of the task increases. Changes in task complexity involving the addition of more items to be remembered or adding a new dimension appears to increase the involvement of NMDA receptors in the hippocampus. Deficits do not appear to be a function of sensory-motor dysfunction, because PCP does not disrupt the acquisition of an object discrimination task.

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