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Intrahippocampal Injections of Phencyclidine but not Naloxone Disrupt Acquisition of a Spatial Continuous Recognition Memory Task

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KESNER, R. P. AND M. DAKIS. *Intrahippocampal injections of phencyclidine but not naloxone disrupt acquisition of a spatial continuous recognition memory task.* PHARMACOL BIOCHEM BEHAV **56**(1) 97–101, 1997.—Rats with 36 nM or 54 nM of phencyclidine (PCP), 36 nM of naloxone or saline injected into the dentate gyrus of the hippocampus were tested for acquisition of a spatial continuous recognition memory task. Results indicate that relative to controls and rats with 36 nM of PCP or 36 nM of naloxone injections, rats with 54 nM of PCP injections were impaired in acquisition of the task across all lags as measured by increases in latency for repeated items. Since it is assumed that successful learning of this continuous recognition memory task depends upon processes associated with consolidation of new learning into long term memory, it appears that high doses of PCP, but not naloxone are sufficient to impair this process. **Copyright © 1997 Elsevier Science Inc.**

Phencyclidine Naloxone Acquisition Retention Short-term memory Consolidation Continuous recognition memory

PHENCYCLIDINE (PCP) is an important pharmacological agent not only because it is a drug of abuse, but also because of its central nervous system action as a competitive antagonist of the *N*-methyl-D-aspartate (NMDA) subtype of excitatory glutamate receptor (12,22). The NMDA receptor has been proposed to be critically involved in mediating long term potentiation (LTP), a phenomenon hypothetically linked to memory (20,27). It is known that there is a high density of PCP receptors in the hippocampus, that PCP blocks LTP in the CA_1 and dentate regions of the hippocampus (3,15,32,33) and that PCP produces performance deficits on learning and memory tasks that are sensitive to hippocampal dysfunction (6,9,11,16,19,24). The exact nature of PCP-induced learning and memory deficit has not yet been determined. Previous studies have demonstrated that low doses of PCP (2–4 mg/kg) disrupt consolidation of new learning into long-term memory, but do not markedly interfere with processes associated with maintaining new or previously learned information within a relatively short time frame (seconds–minutes) (6,11,15,24,29). For example, a low dose of PCP does not alter the rat's ability to acquire a reversal task, but disrupts its ability to remember the reversal learning 24 h later (11). Similarly, low doses of PCP do not alter performance within an 8 arm radial maze, but do affect the ability to remember previous exposure to 4 of 8 arms after a fifteen minute delay with increases in errors in performing the remaining 4 arms (6). Also, low doses of PCP do not alter short-term memory for nonspatial cues in a delayed matching-to-sample task (29) and impair acquisition in a spatial navigation task (dry-land version of water maze) between days (24 hrs) but not within days (minutes) (18). Finally, in a somewhat different study it was shown that peripheral injections of 4 mg/kg PCP also disrupt the acquisition of a continuous recognition memory for spatial location task (16).

Thus, peripheral injections of PCP appear to have specific effects on long-term memory consolidation processes during acquisition of new learning, but appear to have only small effects on short-term or working memory during acquisition of a new task or performance of a previously learned task. Peripheral injections of PCP, however, do not address the question of site of action of PCP within the central nervous system.

Several lines of evidence point to the hippocampal formation, amygdala and neocortex as major sites of action of PCP.

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Specific receptors for PCP are located in the hippocampal formation, amygdala and neocortex (e.g., frontal and parietal cortex), brain regions thought to be important in memory processes (26,30,33,34). The neural distribution of [^{3}H]PCP, [^{3}H]TCP, and NMDA labeled ^{3}H-glutamate binding sites are rather similar with very high levels in the hippocampal formation and relatively high levels in the amygdala and neocortex, including parietal cortex (21,31,34). More specifically within the hippocampus the highest regions include the dentate gyrus and CA_1 regions (14,21,31). Since the hippocampus is likely to be a major site of PCP action and because lesions of the hippocampus produce profound deficits in a continuous spatial recognition memory task (13), it was deemed important to assess the effects of various doses of PCP injected directly into the hippocampus in consolidation of new spatial information using the same continuous recognition memory paradigm used by Kesner and Dakis (16). In this task it is possible to vary list length (lag) and time within a short time frame, so that it should be possible to examine the effects of intracranial injections of PCP into the dentate gyrus on learning of the continuous recognition memory task which would require consolidation of spatial information into long-term memory. In a previous study it was shown that PCP injections into the dentate gyrus of the hippocampus disrupted long-term but not short-term memory in a spatial navigation task (17).

It has also been shown that there are opiate receptors in the dentate gyrus and CA_3 regions of the hippocampus that mediate a form of LTP triggered by high frequency stimulation of the lateral perforant path and mossy fibers (4). Furthermore, naloxone blocks both the lateral perforant path triggered LTP in the dentate gyrus (5) and the mossy fiber triggered LTP in the CA_3 region (8,23). This form of LTP is different from the NMDA receptor mediated LTP. It was, thus, of interest to compare the effects of naloxone which inhibits opiate induced LTP with the effects of PCP which inhibits NMDA induced LTP within the same continuous recognition memory task. It should be noted that in previous maze learning tasks peripheral injections of naloxone improved memory (7,10), but in another study there was no improvement (2). However, in none of these studies was naloxone injected directly into the hippocampus.

METHODS

Subjects

Thirty-eight male Hooded Long–Evans rats received from Simonson, initially weighing 275–350 grams at the start of the experiment, were used as subjects. They were housed individually in standard stainless steel cages in a large, well lit laboratory room and were maintained on a 14/10 hour light/dark schedule. All animals were placed on food deprivation with ad lib water and maintained at 85% of ad lib weight throughout the experiment.

Apparatus

The apparatus consisted of a 12-arm radial maze, constructed of painted white wood, and raised 91.0 cm above the floor. It was kept in a well-lit room with no windows, one door, and 8 calendar pictures placed upon the walls around the room. The center platform was 67.0 cm in diameter and the 12 arms radiating from it were accessible through a clear Plexiglas door at the entrance to each arm. The arms were 65.0 cm long by 9.0 cm wide, and were attached to the central platform with metal braces. Each arm had clear Plexiglas sides, 0.3 cm thick, rising 5.5 cm above the floor of the arm, and extending from the distal end of the arm to 2.5 cm from the central platform. A food well was located at the distal end of each arm, and was 2.56 cm in diameter and 1.5 cm deep. The guillotine door at the juncture of each arm and the central platform was 10.0 cm wide, and when in the closed position, extended 25.5 cm above the surface of the platform. Clear Plexiglas filled the gaps between the doors on the central platform, effectively forming a cylindrical chamber on the platform into which the animals were placed through the open top. The doors could be raised or lowered via a series of pulleys and strings from an adjacent lab room. Each door could be raised an additional 7.5 cm which exposed to view a round, black dot, 1.5 cm in diameter, and 5.0 cm above the floor surface, to serve as an orienting cue.

Surgery

All rats were anesthetized with 40 mg/kg of Nembutal and received a bilateral surgical implantation of 25 gauge guide cannulae, containing a 33 gauge wire stylet which ran the entire length of the outer cannulae. The cannulae were beveled at the ends and the stylet protruded between the beginning and termination of the .5 mm bevel. The cannulae were placed bilaterally in the dentate gyrus region of the hippocampus using the following coordinates: 3.5 mm from bregma, 2.2 mm lateral, and 4 mm ventral (measured from the skull). Following the placements of the cannulae, dental cement was used to anchor the cannulae to two skull screws. In addition, a crown of copper tubing (11 mm inside diameter, 1 mm thick, 8 mm tall) was placed on top of the skull in order to surround and protect the two cannulae. All rats were allowed at least 7 days to recover from surgery prior to behavioral testing.

Procedure

Pretraining involved one session per day for 9 days in which each rat was placed on the center platform of the 12-arm radial maze, with the doors to the arms open. Each food well located at the end of the arms, contained one-quarter piece of Froot Loop cereal, and this reinforcement was not replaced within a session. The animals were allowed 10 minutes to find and eat all the reinforcements. During the last four sessions of this phase of pretraining the Plexiglas doors were closed as the rat exited each arm so that the rat could not reenter each arm. After the ninth session, the second phase of pretraining involved shaping the rat to orient to the cue on the Plexiglas doors. The rat was placed on the center platform with all of the doors in the raised (closed) position, and the experimenter controlled which arm was presented at any given time. Presentation of an arm involved raising the closed door until the black cue dot was visible, and quickly lowering it (opening the door) when the rat oriented to the cue. All twelve arms were randomly presented on each session for 9 additional days, following which training began. The animals were then randomly divided into 4 groups. It is assumed that performance in this task is based on spatial cues and not odor cues based on previous experiments where rotation of the maze did not affect performance.

Ten minutes prior to testing rats were injected with 1μ isotonic saline solutions containing 36 nM PCP (N = 9), 54 nM PCP (N = 10), 36 nM naloxone (N = 8), or saline (N = 11) centrally via the cannulae placements. The stylet plug was removed with tweezers, and a 33 gauge injection cannula was lowered into the guide cannula the same distance as the length

of its particular plug or stylet. The injections were delivered with a picospritzer II pressure ejector and injection time was 30–50 msec. The inner cannula was left in place for 5 sec and the stylet was reinserted immediately afterwards. It was decided to use this short duration injection procedure based on the observation that this procedure with colchicine produced limited lesions of the dentate gyrus (25) and in preliminary data using autoradiography to determine the spread of PCP following PCP injections into the dentate gyrus, it was noted that PCP spreads dorsally into the CA_1 region anterior-posteriorly about 1mm and laterally .8mm leaving CA_3 intact. Phencyclidine was obtained from NIDA.

Each daily training session consisted of placing a rat on the central platform and then allowing it sequential access to a set of 12 arms of the maze. Each arm was cued by the black dot, and as soon as the animal had oriented to the door it was opened by lowering it. The amount of time that elapsed between opening the door and the rat reaching the end of the arm was measured (the maximum latency was 10 sec; if an animal did not start down an arm within 10 sec to completely traverse the arm, the score used for analyses was 10 sec). Of the twelve arm presentations, three or four were arms that had already been presented during that session (repeated arms). Repeated arms were presented with lags ranging from 0 to 6, where a lag of 0 indicates that the arm was repeated immediately after the first presentation, and a lag of 6 indicates that there were six different arm presentations between the first and the repeated presentation. The first time an arm was presented it contained a quarter-piece of Froot Loop cereal for reinforcement; repeated presentations were not reinforced. Which arms were presented, and where they occurred in the random sequence of arm presentations, was counterbalanced across a block of 16 sessions. All rats received a block of 16 sessions which contained 8 instances of each lag. There was only one session per day.

Histology

After completion of the task, each rat was injected with 1μ of 5% Chicago blue dye, 10 minutes later the rat was sacrificed with an overdose of Nembutal and the brain was removed, frozen, sectioned and stained with neutral red in order to determine the spread of the dye, the location of the cannulae, and whether the cannulae were still patent.

RESULTS

The effects of PCP, naloxone or saline injections into the dorsal hippocampus on the acquisition of the continuous recognition task [mean latency (sec)] are shown as a function of lag in Figs. 1 & 2. Also in Figs. 1 & 2, mean latency for the first and second presentation of specific arms as a function of PCP or saline injections across lag is shown. The data indicate that rats inhibit responding (remember the first arm) as a function of lag only when they received saline, 36 nM of PCP or naloxone injections, but do not remember well the first arm presentation under the influence of 54 nM of PCP. A three way analysis of variance for groups (drug treatment) as the between factor and lag and first vs. second presentation as the within factors revealed a significant drug effect ($F = 4.78$, df = 3, 3, $p < .007$), a significant lag effect ($F = 39.6$, df = 6, 204, $p < .0001$), a significant first vs. second presentation effect ($F = 138.9$, df = 1, 34, $p < .0001$), a significant drug by lag interaction ($F = 2.0$, df = 18, 204, $p < .01$), a significant lag by first vs. second presentation interaction ($F = 39.4$, df = 6, 204, $p < .0001$) and finally a significant triple interaction among all three factors ($F = 1.78$, df = 18, 204, $p < .05$). Post hoc Newman Keuls tests revealed that only for the second presentation and lags 0, 1, 2 and 3 was the 54 nM PCP significantly different from the other three groups ($p < .01$). Furthermore, all groups performed better (longer latencies) for the second presentation for the early lags compared to the later lags ($p < .05$). Thus, the data suggest that the 54 nM of PCP

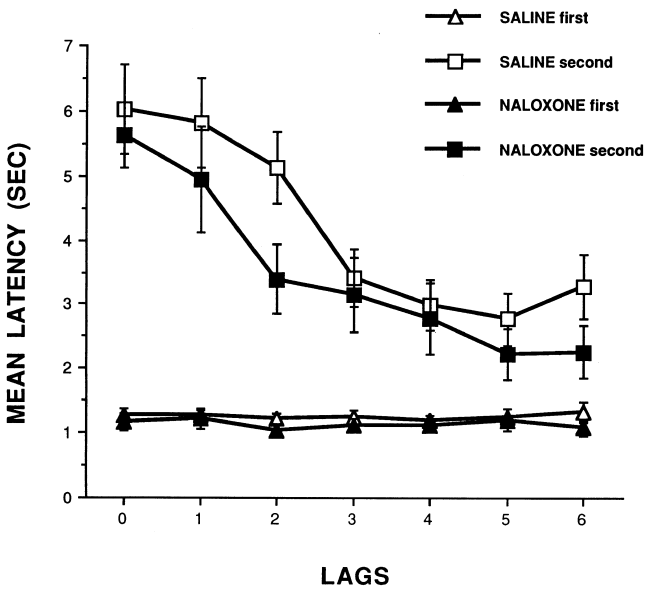

FIG. 1. Mean latency for the first and second presentation as a function of lag for rats with saline or 36 nM of naloxone injected into the dentate gyrus of the dorsal hippocampus during acquisition of the continuous recognition memory task.

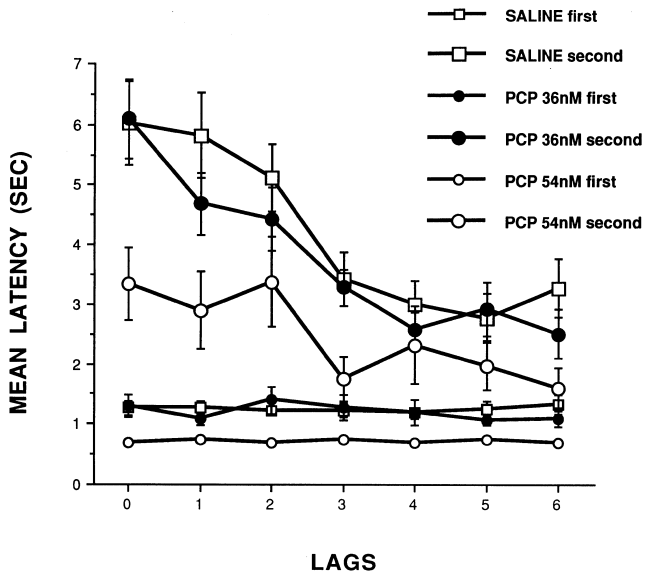

FIG. 2. Mean latency for the first and second presentation as a function of lag for rats with saline, 36 or 54 nM of PCP injected into the dentate gyrus of the dorsal hippocampus during acquisition of the continuous recognition memory task.

group differs from the 36 nM PCP, 36 nM naloxone and control groups across the early lags and only for the second but not the first presentation of a specific arm. The location of the bilateral cannulae placements are shown in Fig. 3A–D for each group and indicate that the cannulae for each of the groups were distributed across the dentate gyrus within the dorsal hippocampus. It was observed that the distribution of the blue dye included most of the dorsal dentate gyrus region and included spread into the CA_1 region and in some animals spread into the cortex dorsal to the dorsal hippocampus.

DISCUSSION

The results of the present study indicate that intracranial injections of PCP into the dentate gyrus of the dorsal hippocampus produce a dose-dependent disruption in the acquisition of a continuous spatial location recognition memory task. This impairment was observed only for the 54 nM PCP injections relative to the 36 nM PCP or saline vehicle injections. These results are similar to what was observed with 4 mg/kg PCP injections (i.p.) using the same task and procedure (16). Even though there was no formal assessment of sensory and motor impairments following intracranial PCP injections, reduced motor activity and inability to negotiate the maze observed with peripheral injections of PCP, were not observed. Furthermore, the presence of a gradient across lags also argues against nonspecific activity effects.

The finding that 54 nM of PCP injected into the dentate gyrus disrupted acquisition performance suggests that PCP disrupts consolidation of new learning into long term memory. These results are consistent with the observation of Morris (15), who demonstrated that AP-5, an NMDA antagonist that blocks NMDA mediated LTP, disrupts consolidation of a spatial navigation task in a water maze. Furthermore, the efficacy of AP-5 appears to be a direct function of the degree of presumed consolidation that is required in that AP-5 was not effective in disrupting consolidation when this process was minimized by providing animals with extensive pretraining experiences (1).

To what extent could these effects have been due to PCP's blockade of NMDA receptors in the hippocampus? Since PCP has been shown to block LTP initiated in the CA_3 region and recorded in the CA_1 region in "in vitro" and "in vivo" preparations (3,32), it is important to point out that it is assumed that LTP induced in the hippocampus represents a central mechanism of information storage associated with the acquisition of new information and perhaps working memory as well (25). However, the effects of intrahippocampal injections of PCP on high frequency stimulation of the Schaffer collateral-induced LTP in CA_1 or perforant path-induced LTP in dentate gyrus in an in vivo preparation has not yet been published. Preliminary work has indicated that doses of PCP used in this study that disrupt consolidation are sufficient to block LTP in the dentate gyrus induced via stimulation of the perforant pathway in an in vivo preparation. Thus, there is a high likelihood that the inhibitory action of PCP on the NMDA receptor is mediated in part by blockade of LTP. This further supports the assumptions that LTP in the hippocampus may play a role in consolidation of new information.

Intracranial injections of naloxone did not produce any disruptive effects on the acquisition of the continuous recognition memory task. Higher doses could not be used because in preliminary studies repeated injections of naloxone resulted in the development of seizure activity. This finding is consistent with one study (2, that reported that naloxone had no effects on maze learning, but is not consistent with other studies demonstrating that naloxone produced facilitatory effects on maze learning (7,10). It should be noted that in the above mentioned studies peripheral rather than direct injections into the dentate gyrus were employed making it difficult to compare the present results with previous studies. In summary, there appears to be a dissociation between the effects of PCP and naloxone injections into the dentate gyrus of the hippocampus in that PCP, but not naloxone, mediates the acquisition (consolidation) of recognition memory for spatial information.

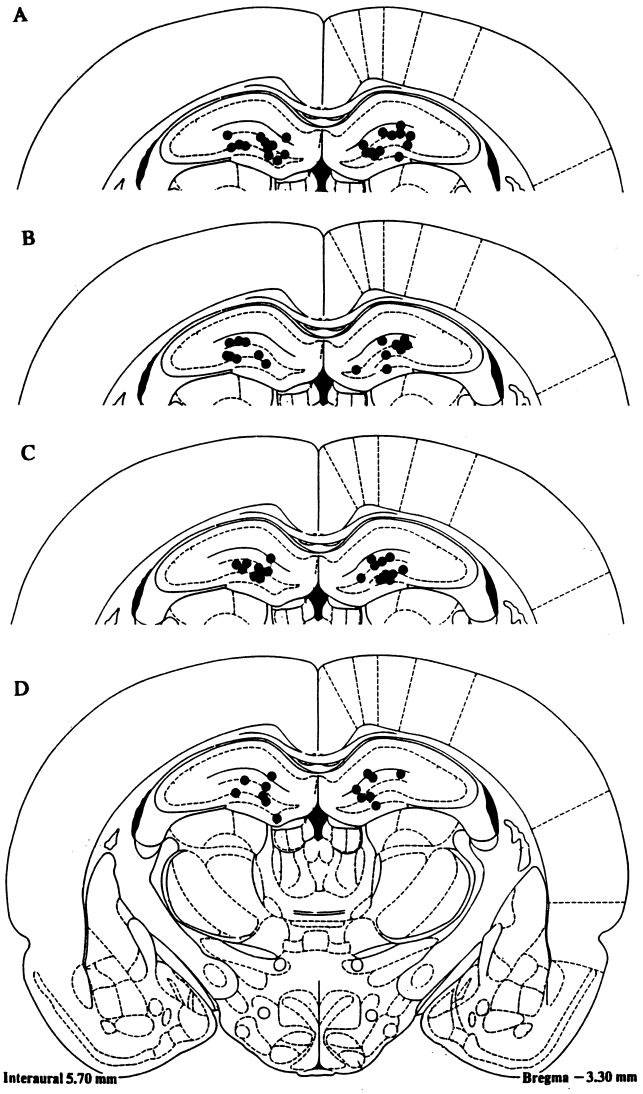

FIG. 3. Distribution of cannulae placements within the dentate gyrus of the dorsal hippocampus for the (A) saline control group, (B) 36 nM PCP group, (C) 54 nM PCP group, and (D) 36 nM naloxone group. The locations are mapped with reference to the Paxinos and Watson rat atlas (28).

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REFERENCES

1. Bannerman, D. M.; Good, M. A.; Butcher, S. P.; Ramsay, M.; Morris, R. G. M. Distinct components of spatial learning revealed by prior training and NMDA receptor blockade. Nature 378:182–186; 1995.
2. Beatty, W. W. Opiate antagonists, morphine and spatial memory in rats. Pharmacol. Biochem. Behav. 19:397–401; 1983.
3. Bourne, G. W.; Capek, R.; Esplin, B. Phencyclidine suppresses hippocampal long-term potentiation through stereospecific activation of phencyclidine receptors. Neuropharmacology 28:49–56; 1989.
4. Bramham, C. R. Opioid receptor dependent long-term potentiation: Peptidergic regulation of synaptic plasticity in the hippocampus. Neurochem. Int. 20:441–455; 1992.
5. Bramham, C. R.; Errington, M. L.; Bliss, T. V. P. Naloxone blocks the induction of long-term potentiation in the lateral but not in the medial perforant pathway of the anesthetized rat. Brain Res. 449:352–356; 1988.
6. Butelman, E. R. The effect of NMDA antagonists in the radial arm maze task with an interposed delay. Pharmacol. Biochem. Behav. 35:533–536; 1989.
7. Canli, T.; Cook, R. G.; Miczek, K. A. Opiate antagonists enhance the working memory of rats in the radial maze. Pharm. Biochem. Behav. 36:521–525; 1990.
8. Derrick, B. E.; Martinez, J. L., Jr. A unique, opioid peptide-dependent form of long-term potentiation is found in the CA_3 region of the rat hippocampus. Advances in the Biosciences 75:213–216; 1989.
9. Ericson, E.; Ahlenius, S. Phencyclidine-induced disruption of an adversely motivated two-choice successive discrimination in the rat. Psychopharmacology (Berl.) 102:171–174; 1990.
10. Gallagher, M.; King, R. A.; Young, N. B. Opiate antagonists improve spatial memory. Science 221:975–976; 1983.
11. Handelmann, G. E.; Contreras, P. C.; O'Donohue, T. L. Selective memory impairment by phencyclidine in rats. Eur. J. Pharmacol. 140:69–73; 1987.
12. Honey, C. R.; Miljikovic, Z.; MacDonald, J. F. Ketamine and phencyclidine cause a voltage-dependent block of responses to L-aspartic acid. Neurosci. Let. 61:135; 1985.
13. Jackson-Smith, P.; Kesner, R. P.; Chiba, A. A. Continuous recognition of spatial and nonspatial stimuli in hippocampal lesioned rats. Beh. Neur. Biol. 59:107–119; 1993.
14. Jarvis, M. F.; Murphy, D. E.; Williams, M. Quantitative autoradiographic localization of NMDA receptors in rat brain using (^{3}H)TCP binding sites. Eur. Journ. Pharmacol. 141:149–152; 1987.
15. Kesner, R. P. Phencyclidine blocks high frequency stimulation of prefrontal path induced LTP in the dentate gyrus (unpublished observations).
16. Kesner, R. P.; Dakis, M. Phencyclidine disrupts acquisition and retention performance within a spatial continuous recognition memory task. Pharmacol. Biochem. Behav. 44:419–424; 1993.
17. Kesner, R. P.; Dakis, M. Phencyclidine injections into the dorsal hippocampus disrupt long- but not short-term memory within a spatial learning task, Psychopharmacologia. 120:203–208; 1995.
18. Kesner, R. P.; Dakis, M.; Bolland, B. Phencyclidine disrupts long- but not short-term memory within a spatial learning task. Psychopharmacology, 111:85–90; 1993.
19. Kesner, R. P.; Hardy, J. D.; Novak, J. M. Phencyclidine and behavior: II. Active avoidance learning and radial arm maze performance. Pharmacol. Biochem. Behav. 18:1057–1063; 1983.
20. Lynch, G; Baudry, M. The biochemistry of memory: a new and specific hypothesis. Science 244:1057–1063; 1984.
21. Maragos, W. F.; Penney, J. B.; Young, A. B. Anatomic correlation of NMDA and ^{3}H-TCP-labeled receptors in rat brain. J. Neurosci. 8:493–501; 1988.
22. Martin, D.; Lodge, D. Ketamine acts as a non-competitive *N*-methyl-D-aspartate antagonist on frog spinal cord in vitro. Neuropharmacology 24:99; 1985.
23. Martin, M. R. Naloxone and long-term potentiation of hippocampal CA_3 field potentials in vitro. Neuropeptides 4:45–50; 1983.
24. McCann, D. J.; Winter, J. C. Effects of phencyclidine, N-Allyl-N-normetazocine (SKF-10,047) and verapamil on performance in a radial maze. Pharmacol. Biochem. Behav. 24:187–191; 1986.
25. McNaughton, B. L.; Barnes, C. A.; Meltzer, J.; Sutherland, R. J. Hippocampal granule cells are necessary for normal spatial learning but not for spatially-selective pyramidal cell discharge. Exp. Brain Res. 76:485–496; 1989.
26. Monaghan, L. T.; Cotman, C. W. Contribution of *N*-methyl-D-aspartate-sensitive L-[^{3}H]Glutamate-binding sites in the rat brain. J. Neurosci. 5:2909–2919; 1985.
27. Morris, R. G. M. Synaptic plasticity and learning: selective impairment of learning in rats and blockade of long-term potentiation in vivo by the *N*-methyl-D-aspartate receptor antagonist, AP5. J. Neurosci. 9:3040–3057; 1989.
28. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. 2nd ed. Academic Press, Orlando, Fla., 1986.
29. Pontecorvo, M. J.; Clissold, D. B.; White, M. F.; Ferkany, J. W. *N*-methyl-D-aspartate antagonists and working memory performance: Comparison with the effects of scopolamine, propranolol, diazepam, and phenylisopropyladenosine. Behav. Neurosci. 105:521–535; 1991.
30. Quirion, R.; Hammer, R. P.; Herkenham, M.; Pert, C.B. Phencyclidine (angel dust)/sigma 'opiate' receptor: Visualization by tritium-sensitive film. Proc. Nat. Acad. Sci. USA 78:5881; 1981.
31. Sircar, R.; Zukin, S. R. Quantitative localization of [^{3}H]TCP binding in rat brain by light microscopy autoradiography. Brain Res. 344:142–145; 1985.
32. Stringer, J.; Guyenet, P. G. Elimination of long-term potentiation in the hippocampus by phencyclidine and ketamine. Brain Res. 258:159–164; 1983.
33. Vincent, J. P.; Kartalovski, B; Geneste, P; Kamenka, J. M.; Lazdunski, M. Interaction of phencyclidine ('angel dust') with a specific receptor in rat brain membranes. Proc. Nat. Acad. Sci. USA 76:4678–4682; 1979.
34. Zukin, S. R.; Fitz-Syage, M. L.; Nichtenhauser, R.; Zukin, R. S. Specific binding of [^{3}H] Phencyclidine in rat central nervous tissue: Further characterization and technical considerations. Brain Res. 258:277–284; 1983.