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N-Methyl-D-Aspartate Receptors in Associative Eyeblink Conditioning: Both MK-801 and Phencyclidine Produce Task- and Dose-Dependent Impairments[1]

LUCIEN T. THOMPSON and JOHN F. DISTERHOFT

Department of Cell and Molecular Biology and the Institute for Neurosciences, Northwestern University Medical School, Chicago, Illinois

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ABSTRACT

The effects of N-methyl-D-aspartate receptor blockade on two major variants of rabbit eyeblink conditioning were evaluated using a selective noncompetitive antagonist, [5R, 10S]-[+]-5-methyl-10,11-dihydro-5H-dibenzo[a, d] cyclo-hepten-5,10-imine hydrogen maleate; dizocilpine (MK-801) or phencyclidine (PCP), a drug of abuse. Either MK-801 or PCP (given daily) impaired rabbits' ability to associate tone conditioned stimuli with airpuff unconditioned stimuli, with the severity of impairment exhibiting clear dose and task dependencies. Trace-conditioned rabbits given $\geq$ 80 μg/kg of MK-801 or $\geq$1.0 mg/kg of PCP failed to reach a criterion of 80% conditioned responses during training, with significant impairments seen at intermediate doses. Delay-conditioned rabbits, although dose-dependently slowed, successfully acquired the task, even when given doses of MK-801 or PCP that completely blocked acquisition in trace conditioning. Additionally, even low doses of MK-801 (10 μg/kg) or of PCP (0.1 mg/kg) severely altered conditioned response timing in trace but not in delay conditioning, resembling effects observed after hippocampal lesions. Doses of MK-801 or PCP that impaired acquisition also severely impaired extinction of both trace- and delay-conditioned eyeblink responses. However, neither MK-801 nor PCP altered retention or timing of previously learned responses. Higher doses of MK-801 ($\geq$200 μg/kg) or of PCP ($\geq$2.0 mg/kg) dose-dependently impaired unconditioned response performance, although lower doses of MK-801 ($\leq$160 μg/kg) or of PCP ($\leq$1.0 mg/kg) had no effects on unconditioned responses or on nonassociative pseudoconditioned responses. The deficits observed indicate that although not necessary for retention, N-methyl-D-aspartate receptor activation may facilitate acquisition of delay-conditioning. N-methyl-D-aspartate receptor activation appears to be necessary for acquisition of trace conditioning, and for extinction in either paradigm.

The NMDA receptor/channel-complex is a transmembrane neuronal protein containing multiple ligand binding sites and an ionophore with high selectivity for Ca^{++} entry (Collingridge and Lester, 1989). MK-801 and other noncompetitive NMDA antagonists bind to a site located within the receptor's ionophore, binding that requires the channel be in an open state to block it (Honey *et al.*, 1985). This use-dependent block of the activated NMDA receptor has made noncompetitive antagonists such as MK-801 valuable tools for investigating the functional role of the NMDA receptor in learning.

Excitatory amino acid receptors play necessary permissive roles in several persistent forms of neural plasticity (Watkins and Collingridge, 1989; Meldrum *et al.*, 1991). LTP is a model of synaptic plasticity first demonstrated in the hippocampal circuit (Bliss and Gardner-Medwin, 1971). Interest in NMDA receptors' role in plasticity and learning was sparked by demonstrations that NMDA receptors are involved in induction, but not expression, of LTP in both dentate and CA1 regions of the hippocampus (Collingridge *et al.*, 1983). NMDA receptors are highly enriched in the hippocampus (Monaghan and Cotman, 1985), but are found in many brain regions, including the cerebellum (Sekiguchi *et al.*, 1987; Yi *et al.*, 1988). Morris *et al.* (1986) demonstrated that competitive antagonism of NMDA receptors with AP5 not only prevents induction of LTP in the hippocampus but also severely impairs acquisition of hippocampally dependent spatial learning tasks in the same animals. Numerous studies have shown that competitive NMDA antagonists inhibit acquisition but not previously learned performance of other behavioral tasks (*e.g.,* Laroche *et al.*, 1989; Staubli *et al.*, 1989; Morris *et al.*, 1990; Fanselow *et al.*, 1994). Other work has

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ABBREVIATIONS: NMDA, N-methyl-d-aspartate; MK-801, ([5R, 10S]-[+]-5-methyl-10,11-dihydro-5H-dibenzo[a, d] cyclo-hepten-5,10-imine hydrogen maleate; dizocilpine); PCP, phencyclidine (1-[1-phencyclohexyl] piperidine); LTP, long-term potentiation; AP5, D-2-amino-phosphono-pentanoate; NMR, nictitating membrane response; CS, conditioned stimulus; US, unconditioned stimulus; CR, conditioned response; UR, unconditioned response; NZW, New Zealand white; ISI, interstimulus interval.

shown that noncompetitive NMDA antagonists also impair acquisition but not retention of spatial-learning tasks (Heale and Harley, 1990; Shapiro and Caramanos, 1990; McLamb *et al.*, 1990). Conversely, Thompson *et al.* (1992) demonstrated that glycine coagonists for the NMDA receptor dramatically increase learning rate in 500 msec trace-conditioning of an eyeblink or NMR.

Rabbit eyeblink conditioning has served as a model system for analyses of the neural substrates of learning (Disterhoft and Buchwald, 1980; Schindler and Harvey, 1990; Thompson *et al.*, 1976), with two major variants, *trace-* and *delay*-conditioning, widely recognized (Gormezano *et al.*, 1987). The long-interval (500 msec) trace-conditioned task requires intact hippocampal function for successful acquisition (Moyer *et al.*, 1990; Solomon *et al.*, 1986). In trace-conditioning, a stimulus-free "trace" interval intervenes between conditioned stimulus offset and unconditioned stimulus onset, forcing the subject to form a very short-term memory of the CS to successfully predict US onset and therefore perform CRs appropriately timed to avoid the aversive US. In delay-conditioning, the CS and US overlap temporally, although CS onset precedes US onset and the interstimulus interval (ISI) between CS and US onset is used to define the paradigm. Recent work with young rabbits (Thompson *et al.*, 1996a; Solomon and Groccia-Ellison, 1996) showed that trace-conditioned tasks using a 600 msec ISI were more slowly acquired than delay-conditioned tasks using a 600 msec ISI, while delay-conditioned tasks comparing ISIs of 250–600 msec or 400–900 msec were acquired at comparable rates. Hippocampal lesions block acquisition of trace-conditioning, and also severely disrupt the timing of the relatively few eyeblink responses observed within the trace interval (Moyer *et al.*, 1990; Solomon *et al.*, 1986). In the absence of a functional hippocampus, temporal processing may be carried out, albeit within certain limited stimulus parameters (*e.g.* with stimulus overlap, as in delay-conditioning, or with very short–≤ 300 msec–trace intervals), elsewhere in the brain (Schmaltz and Theios, 1972; Akase *et al.*, 1989; Moyer *et al.*, 1990).

Studies with daily PCP treatment (Kesner *et al.*, 1983; McCann *et al.*, 1986; Handelmann *et al.*, 1987) have also shown dose-dependent impairments of acquisition in spatial tasks that are severely impacted by hippocampal damage. PCP has also been shown to have activity as a noncompetitive antagonist of NMDA receptors (Fagg, 1987; Honey *et al.*, 1985; O'Shaughnessy and Lodge, 1988). If this were the only action of PCP, it would be reasonable to hypothesize that phencyclidine would produce behavioral effects similar to those of MK-801. However, PCP also interacts with other receptors (Gundlach *et al.*, 1985; Domino, 1986), particularly at high doses. One goal of our study was to determine whether learning deficits produced by phencyclidine could be separated from other nonassociative behavioral effects, and if so, whether these associative effects were mediated via PCP's antagonism of the NMDA receptor.

As with MK-801 and other noncompetitive NMDA antagonists, PCP binds to a site located within the NMDA receptor's ionophore, and requires that this channel be in an open state to block it (Honey *et al.*, 1985). PCP or MK-801 injected locally into the hippocampus produced similar deficits in learning of but not in performance of previously learned spatial tasks (Kesner and Dakis, 1995). PCP's effects on hippocampal neurons have been studied in intact animals and *in vitro*, and hippocampal synaptic plasticity has been proposed as a model for the neural plasticity underlying learning (Andersen, 1987; Matthies, 1989; Morris *et al.*, 1990). PCP dose-dependently reduces synaptically evoked extracellular field potential population spikes and blocks one form of plasticity, synaptic LTP *in vivo* and *in vitro* in both the CA1 region (Stringer and Guyenet, 1982, 1983; Coan and Collingridge, 1987) and dentate gyrus (Desmond *et al.*, 1991) of the hippocampus. Although LTP has frequently been cited as a model of associative learning (see extended discussion in Barnes, 1995), direct links between associative learning and LTP have not been definitively demonstrated in vertebrates and remain an area of intense study. In fact, Robinson (1993) demonstrated that low doses of MK-801 (50-100 μg/kg daily) that effectively slowed acquisition of delay-conditioned eyeblink responses were without effect on potentiation of hippocampal perforant path synaptic responses, suggesting that LTP and acquisition may not be directly linked in this learning task.

The experiments described here were designed to test the hypothesis that NMDA receptors are active in the acquisition of associative eyeblink conditioning. If NMDA receptor activation is required for acquisition of conditioned eyeblinks, the selective noncompetitive antagonist MK-801, a dibenzocycloheptenimine that blocks the activated ionophore of the NMDA receptor, should also block acquisition of both trace and delay conditioning. If NMDA receptor activation is not required in both tasks, differential results might be obtained. If PCP's primary effect in learning is to block NMDA receptors, its effects might mimic those seen with MK-801 across a specified range of doses. The effects of MK-801 or PCP on previously acquired conditioned responses were also examined, using two different postacquisition procedures, and additional pseudoconditioning control procedures were included to test for nonassociative effects of NMDA blockade on expression of the NMR. The effects of daily treatment with MK-801 or with PCP on both trace and delay eyeblink conditioning have not been previously compared. Our study thus tested MK-801 and PCP's effects across a wide range of doses, and assessed effects not only on acquisition of the conditioned eyeblink but also on postacquisition behaviors, examining both extinction and retention of the CR.

Methods

Subjects. The subjects used were 208 young (3-4 mo old, 1.5-2 kg) female NZW rabbits, Oryctolagus cuniculus (Kuiper Rabbitry, Gary, IN or Hazelton Research Products, Denver, PA). All rabbits were experimentally naive before training. The rabbits were bred for experimental use, were housed individually in a general colony room and maintained on a 14/10 hr light/dark schedule with *ad libitum* access to food and water. Rabbits were assigned to groups as described below. All subjects were trained in pairs, with individuals within pairs counterbalanced among groups.

Experimental groups. The acquisition, postacquisition and pseudoconditioning control groups used in these experiments are summarized in table 1. MK-801 and PCP experiments were run separately. Pilot work was also necessary to determine appropriate dose ranges for testing, as rabbit doses for NMDA antagonists are not directly comparable to those of the other major model (rats) available in the behavioral pharmacology literature. In these pilot studies, 16 rabbits that had been previously delay-conditioned (as described below) were used to determine appropriate dose ranges of MK-801 or of PCP that did not affect performance of the UR (and

thus were used to test for specific associative effects). Six rabbits were given daily doses of 120, 160, 200, 240 or 280 μg/kg of MK-801 (RBI; Natick, MA) in 0.9% saline, i.m., in a random counter-balanced fashion, and 10 rabbits were given daily doses of 0.1, 1.0, 2.0, 5.0 or 10.0 mg/kg of PCP (NIDA) in 0.9% saline, i.m., in a random counter-balanced fashion (more rabbits were used for PCP testing, due to deaths of some rabbits given the highest dose of PCP). Each rabbit was tested three times at each dose, and UR performance was assessed for 20 trials both before and for 30 min after MK-801 or PCP administration.

Once doses had been determined that were without effect on the UR, acquisition studies using these and lower doses began. For MK-801 experiments, rabbits each received equivolume daily doses of 0.0, 10, 40, 80 or 160 μg/kg, i.m., of MK-801 in 0.9% saline within 5 min before training. For PCP experiments, rabbits each received equivolume daily doses of 0.0, 0.1 or 1.0 mg/kg of PCP, i.m., in 0.9% saline within 5 min before training. Additional sets of subjects were also pseudoconditioned or were tested postacquisition, as described below, using effective doses of MK-801 or PCP determined empirically from acquisition testing.

Trace and delay conditioning. All training was conducted in a double-blind fashion. Behavioral conditioning experiments were controlled by microcomputers using custom software (Akase *et al.*, 1994). As previously described, all rabbits were surgically fitted with nylon restraining headbolts, were allowed 48 hr for postsurgical recovery and then were habituated to restraint in the training environment (Moyer *et al.*, 1990; Thompson *et al.*, 1992, 1996a, b). Pairs of rabbits were trained in individual sound-attenuated chambers for daily 80-trial sessions, with intertrial intervals averaging 45 sec. The right eyelid was held open with stainless steel eyeclips attached to velcro straps, and nictitating membrane or third eyelid extension responses (NMRs) were measured noninvasively via changes in light reflectance with an optical detector (Thompson *et al.*, 1994). The tone CS used was an 85 dB, 6 kHz pure tone presented via stereo headphones. The corneal airpuff US used for all conditioning and pseudoconditioning was a 150 msec, ~3.0 psi corneal airpuff delivered from a pipette tip placed 3 mm away from the posterior corner of the right cornea, sufficient to elicit a reliable NMR as the UR. A constant US intensity was used for all subjects to allow tests for drug-induced nonassociative differences in somatosensory sensitivity. Only paired CS-US trials were used for both trace- and delay-conditioning studies, to avoid confounds related to CS preexposure or to blocking consequent to unpaired stimulus presentations. In our experience, this design allows a reliable assessment of UR performance across a variety of treatment conditions (*e.g.,* Thompson *et al.*, 1992, 1996a), that can also be compared to results obtained from unpaired pseudoconditioning trials. Unconditioned response characteristics were typically stable after one session of training, and were averaged across all trials for the final session of training, with measures of UR amplitude and latency reported.

Delay-conditioning used a 400-msec duration CS that coterminated with the airpuff US (giving a CS onset to US onset ISI of 250 msec). This same paradigm was earlier used in this laboratory in studies that demonstrated hippocampal lesions have no significant effects on acquisition but block extinction of recently acquired delay-conditioned eyeblinks (Akase *et al.*, 1989), and gave a longer CS alone interval (150 msec) in which to examine response timing issues than if CS duration were strictly matched with that used in trace-conditioning (100 msec). For trace-conditioning, a 100-msec duration tone CS was followed (after a 500-msec trace interval) by the airpuff US (giving a CS onset-US onset ISI of 600-msec). Again, this same paradigm was earlier used in this laboratory in studies that demonstrated hippocampal lesions block acquisition of a trace-conditioned eyeblink task (Moyer *et al.*, 1990). Thus, two different variants of the eyeblink conditioning task that are differentially affected by hippocampal lesions were used.

All subjects were trained to a criterion of 80% conditioned responses (CRs, defined in all experiments as NMRs beginning after CS onset but before US onset) within an 80-trial conditioning session. Training of rabbits continued using daily 80-trial sessions until the rabbit's performance reached 64 conditioned responses within an 80-trial session (hereafter termed 80% CRs) or until a total of 2000 training trials were received if criterion was not reached. Performance was assessed in terms of the number of trials required to reach this rigorous 80% CRs criterion (see Thompson *et al.*, 1996a).

Postacquisition testing. Saline-treated controls that were trace or delay conditioned in the experiments described above, along with additional subjects trained to criterion as described and given daily saline injections, then underwent extinction. After reaching criterion, they were trained for three additional daily sessions, using unpaired CS alone presentations at the same trial intervals as described above. Half were treated daily within 5 min before extinction testing with either 160 μg/kg of MK-801 or 1.0 mg/kg of PCP, the highest doses used that were without effect on the UR, and half

TABLE 1
Subject assignment for acquisition, postacquisition and pseudoconditioning studies

Drug Tested	Doses Tested	Initial Training	Postacquisition Testing	*n* Subjects/Dose
MK-801				
	5	Trace-conditioned		6
	2	Trace-conditioned	Extinction	6[a]
	2	Trace-conditioned	Retention	6
	2	Pseudo-conditioned[b]		5
	5	Delay-conditioned		6
	2	Delay-conditioned	Extinction	6[a]
	2	Delay-conditioned	Retention	6
	2	Pseudo-conditioned[c]		5
PCP				
	3	Trace-conditioned		6
	2	Trace-conditioned	Extinction	6[a]
	2	Trace-conditioned	Retention	6
	2	Pseudo-conditioned[c]		5
	3	Delay-conditioned		6
	2	Delay-conditioned	Extinction	6[a]
	2	Delay-conditioned	Retention	6
	2	Pseudo-conditioned[b]		5

[a] Some of these subjects were saline controls from acquisition experiments, although others were trained to criterion in the same fashion with daily saline injections and used only for postacquisition testing.
[b] These subjects received unpaired 100-msec duration tone CSs.
[c] These subjects received unpaired 250-msec duration tone CSs.

served as saline controls (each experimental rabbit being paired with a control). Thus, measures of the effects of MK-801 or of PCP on extinction of a trace- or delay-conditioned eyeblink response were obtained. Performance was assessed in terms of the number of CRs per session of testing.

Additional groups of rabbits were trained to criterion in either the trace- or delay-conditioning paradigms described above, and received daily pretraining saline injections during acquisition. On 3 days after reaching criterion, these subjects then received an additional three sessions of paired CS-US presentations as during acquisition. Again, each experimental rabbit was paired with a saline control. Experimental rabbits were given daily treatment with either 160 μg/kg of MK-801 or 1.0 mg/kg of PCP. The performance of these subjects assessed retention of the recently acquired trace- or delay-conditioned association in the presence or absence of the NMDA receptor antagonist. Again, performance was assessed in terms of the number of CRs per session of testing.

Pseudoconditioning. Pseudoconditioned control rabbits were used to test for nonassociative effects of MK-801 or of PCP treatment. Half received a 100-msec duration tone CS (used for trace-conditioning) and half a 250-msec duration tone CS (used for delay-conditioning). Each pseudoconditioned subject was paired with a saline control, and experimental subjects received daily treatment with 160 μg/kg of MK-801 or with 1.0 mg/kg of PCP. Pseudoconditioning consisted of explicitly unpaired presentations of 80 CS and 80 US presentations in a pseudorandom interval (10 of each in 20 trials), with the average intertrial interval half that used in conditioning. Pseudoconditioned control animals received 10 daily training sessions of 160 trials each, to insure that pseudoconditioned rabbits received the same number of CSs and USs at the same average rate as paired stimuli were received by conditioned rabbits within a session. Measures of response number, amplitude and latency were assessed and averaged across all trials, and compared with results from paired trials.

Statistical analyses. A drug dosage by task analysis of variance was carried out (Statview II, Abacus Concepts, Berkeley, CA). Behavioral data were then tested for main effects of drug dosage (dose dependence) within either the trace- or delay-conditioning task with one-way analyses of variance. Post tests were carried out with Scheffe's test. A minimal criterion for statistical significance of $P < .05$ was used. Behavioral data are cited as means ± S.E.M.

Results

MK-801 impaired acquisition. Eyeblink conditioning was severely impaired by daily treatment with MK-801 [$F(4, 50) = 16.5$, $P < .0001$]. The impairment was both dose and task-dependent (see fig. 1), with a complete block of trace-conditioning observed at higher doses, although delay-conditioning was only slowed but not blocked even at high doses.

Trace-conditioning was severely impaired by NMDA receptor blockade with MK-801 [$F(4, 25) = 40.6$, $P < .0001$]. Daily doses of 80 μg/kg or more of MK-801 completely blocked acquisition of trace-conditioning, with rabbits failing to learn the task even after 2000 trials of training (controls and rabbits receiving doses of 10 μg/kg of MK-801 daily learned in an average of 433.3 trials). Daily doses of 40 μg/kg of MK-801 significantly slowed but did not block trace conditioning ($P < .003$). The block of learning at doses of 80 μg/kg daily or more was not an artifact of insufficient training being allowed for impaired rabbits to reach criterion (*i.e.,* a possibility that more than 2000 trials would be required for successful acquisition). Analyses of averaged and of individual data for rabbits receiving doses of 160 μg/kg of MK-801 daily, *e.g.,* indicated that the learning curves for these subjects were flat (data not shown). Subjects receiving high doses of MK-801 (80 or 160 μg/kg daily) never exceeded response rates of more than 30% CRs/training session, and typically exhibited rates lower than 20% CRs/session, a rate similar to that seen in pseudoconditioning.

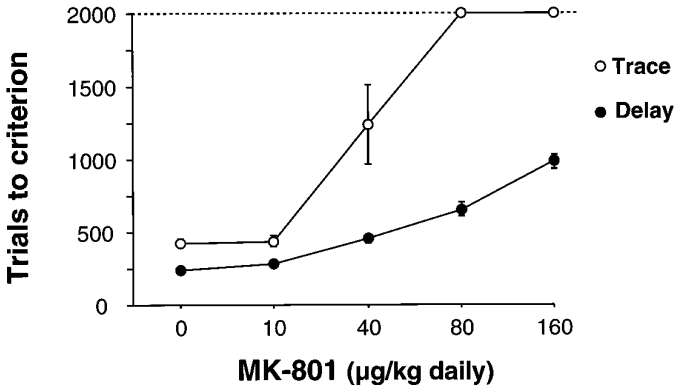

Fig. 1. The learning deficit in acquisition of eyeblink conditioning produced by daily treatment with MK-801 was both dose and task dependent. Doses of MK-801 of 80 μg/kg or more daily blocked acquisition of 500-msec trace-eyeblink conditioning, although doses of 40 μg/kg daily produced intermediate impairments. No rabbits treated with 80 μg/kg or more of MK-801 daily reached behavioral criterion within 2000 training trials in the trace-conditioning task. Delay conditioning was significantly slowed by doses of 40 μg/kg daily or more, with greater impairments at greater doses, but no dose was sufficient to block acquisition. Doses of 200 μg/kg daily or above produced non-associative effects on the UR (see table 1), and were not used for testing associative effects.

Delay-conditioning was also significantly impaired by MK-801 treatment [$F(4, 25) = 75.9$, $P < .0001$]. In delay conditioning, acquisition was significantly slowed at all doses of more than 40 μg/kg, but was never blocked (see fig. 1). This effect was also dose dependent, with greater effects at higher doses. It should be noted that 16 times as much MK-801 daily was required to triple the number of trials required to reach criterion in delay conditioning, although only four times as much was required for the same effect in trace-conditioning.

Nonassociative effects of MK-801 treatment were absent at doses of 160 μg/kg daily or lower (see table 2), although significant associative effects are reported above. No significant effects on UR number, amplitude or latency were noted at doses up to and including 160 μg/kg daily of MK-801 ($P > .4$). Pseudoconditioning also indicated a lack of stimulus sensitization effects across the full range of doses tested ($P > .3$), with infrequent blinks occurring at the same rates, amplitudes and latencies within the 600- or 250-msec interval after CS onset in pseudo-trace or -delay paradigms, respectively. Doses of 200 μg/kg or greater of MK-801 daily, however, produced dose-dependent reductions in the amplitude and increases in the latency of the UR (indicative of reported dissociative anesthetic effects). UR frequency (number of URs per session) was unaffected across the range of doses of MK-801 tested (0.0-280 μg/kg daily).

PCP also impaired acquisition. Daily treatment with phencyclidine significantly impaired acquisition of eyeblink conditioning [$F(5, 30) = 13.2$, $P < .0001$]. This impairment was both dose and task dependent (see fig. 2), with a complete block of trace-conditioning observed at higher doses, although delay-conditioning was only slowed but not blocked even at high doses. Summary learning curves for control and PCP-treated subjects are also shown in Figure 2. These

TABLE 2

Daily treatment with doses of MK-801 up to 160 μg/kg daily had no effects on URs, although learning of both trace- and delay-conditioning was affected at doses of 40 to 160 μg/kg daily.

Training	Dose of MK-801	UR Amplitude (mV)	UR Peak Latency (ms)	CR Frequency (% Within ISI Used for Conditioning)
Trace-conditioned				
	0.0	2998 ± 181	183 ± 41	
	10	3007 ± 105	201 ± 37	
	40	2911 ± 147	194 ± 36	
	80	3003 ± 162	211 ± 32	
	160	2945 ± 158	204 ± 47	
Delay-conditioned				
	0.0	2876 ± 201	193 ± 24	
	10	3092 ± 188	205 ± 23	
	40	2922 ± 139	187 ± 31	
	80	3114 ± 177	201 ± 36	
	120	2990 ± 126	209 ± 38	
	160	2894 ± 118	202 ± 34	
	200	2321 ± 141[b]	231 ± 40	
	240	1961 ± 188[c]	277 ± 43[b]	
	280	1344 ± 219[d]	319 ± 31[c]	
Pseudoconditioned				
	0.0	3014 ± 131	193 ± 37	14.9 ± 5.8
	160	2992 ± 145	204 ± 21	15.3 ± 6.9

[a] With higher doses of MK-801 (200 μg/kg or more daily), dose-dependent anesthetic reductions in UR amplitudes were apparent. Similar observations about a lack of effect on response sensitization were obtained in pseudoconditioning control experiments with doses of MK-801 that impaired learning.
[b] $P < .002$.
[c] $P < .0008$.
[d] $P < .0001$.

curves graphically illustrate the difference in response acquisition observed between groups.

Trace-conditioning was dose-dependently impaired by PCP treatment [$F(2, 15) = 40.6$, $P < .0001$] (see fig. 2). Moderate doses of PCP (1.0 mg/kg daily) blocked acquisition but did not alter the amplitude of the UR ($P < .0001$). Although higher doses of PCP (2.0, 5.0 or 10.0 mg/kg daily) blocked acquisition of trace-conditioning in pilot studies (data not shown), higher doses were also anesthetic, and diminished or eliminated responses to the airpuff in a dose-dependent fashion (see dose-dependent effects of PCP on the UR, in table 2). Low doses of PCP (0.1 mg/kg daily) did not affect the UR, and did not alter the rate of acquisition of eyeblinks within the trace interval (eyeblinks that were, by definition, CRs). Pseudoconditioning also revealed that PCP treatment did not significantly alter responses to unpaired stimuli at doses of 1.0 mg/kg daily (see table 3). Thus, complete block of trace-conditioning occurred at a dose (1.0 mg/kg daily) that produced no discernable effects on the UR either during training or in pseudoconditioning.

Delay conditioning was also significantly impaired by PCP treatment [$F(2, 15) = 29.1$, $P < .0001$]. In delay-conditioning, acquisition was significantly slowed by doses of 1.0 mg/kg or higher, but was not blocked (see fig. 2). It should be noted that acquisition (trials required to reach criterion) was approximately doubled in delay-conditioning by a dose of 1.0 mg/kg of PCP, although complete block of acquisition occurred in trace conditioning at the same dose.

No rabbits receiving a dose of 1.0 mg/kg of PCP reached behavioral criterion in trace-conditioning, although all delay-conditioned rabbits and all trace-conditioned rabbits receiving lower doses of PCP did. Rabbits receiving low doses of PCP (0.1 mg/kg daily) took no longer than control rabbits to reach criterion in either eyeblink task ($P > .45$). The learning impairments produced by PCP were thus both dose- and task-dependent.

MK-801 impaired extinction but not retention. Extinction and retention data from trace- and delay-conditioned subjects were combined, as no significant task-dependent differences in the effects reported here were observed. The behaviorally effective dose of 160 μg/kg daily of MK-801 strongly blocked extinction of both trace- and delay-conditioned eyeblink responses ($P < .01$; see fig. 3a). With MK-801, the number of CRs performed in response to unpaired CSs did not decline over trials, although saline-treated controls given repeated unpaired presentations of the CS exhibited normal extinction to low levels of performance. However, the same dose had no effect on expression of previously learned CRs ($P > .4$; see fig. 3b). Performance continued at or above criterion levels in both MK-801-treated and saline-control groups. As noted above, this high dose of MK-801 was also without effects on the UR. These finding are consistent with reports from other behavioral systems, where new learning was blocked but previously acquired responses were maintained during NMDA receptor blockade (Shapiro and Caramanos, 1990).

PCP also impaired extinction but not retention. Doses of 1.0 mg/kg daily of PCP impaired extinction of learned responses once explicit pairing of the CS and US were ended ($P < .01$). As seen in figure 4a, within three sessions of extinction, response rates for saline-treated controls that had reached criterion fell to rates indistinguishable from those of naive controls (*i.e.,* those of untrained rabbits). Subjects that had acquired the task previously (saline controls); however, continued performing the CR to the unpaired CS at a high rate if treated postacquisition with 1.0 mg/kg of PCP. No significant differences in these effects were observed in comparisons of trace- or delay-conditioned subjects, so data for both tasks were combined. It should be noted that these results are consistent with those obtained using MK-801 treatment in the same tasks. Mean extinction performance curves for PCP-treated and control rabbits are shown in figure 5, and illustrate the clear difference in behavior resulting from treatment with a dose of PCP that was also effective in blocking acquisition of this task.

Conversely, PCP treatment had no effect on retention of the learned eyeblink response when tested using paired CS-US presentations ($P > .6$). Both trace- and delay-conditioned rabbits trained to criterion (treated during acquisition as saline control) continued to perform at criterion levels indistinguishable from that of saline controls, despite daily postacquisition treatment with doses of 1.0 mg/kg of PCP (see fig. 4b). Again, these results are consistent with those obtained using MK-801 treatment in the same tasks, in which postacquisition retention of the conditioned eyeblink response was unaffected by NMDA receptor blockade, irrespective of the training task (trace or delay) tested.

MK-801 altered CR timing in acquisition. Conditioned response timing was significantly altered during acquisition in trace- but not in delay-conditioning, resulting in a temporal shift to short-latency CRs in the trace-conditioned task. Figure 6 shows the leftward shift in CR latencies observed at an intermediate dose (40 μg/kg) of MK-801; similar shifts

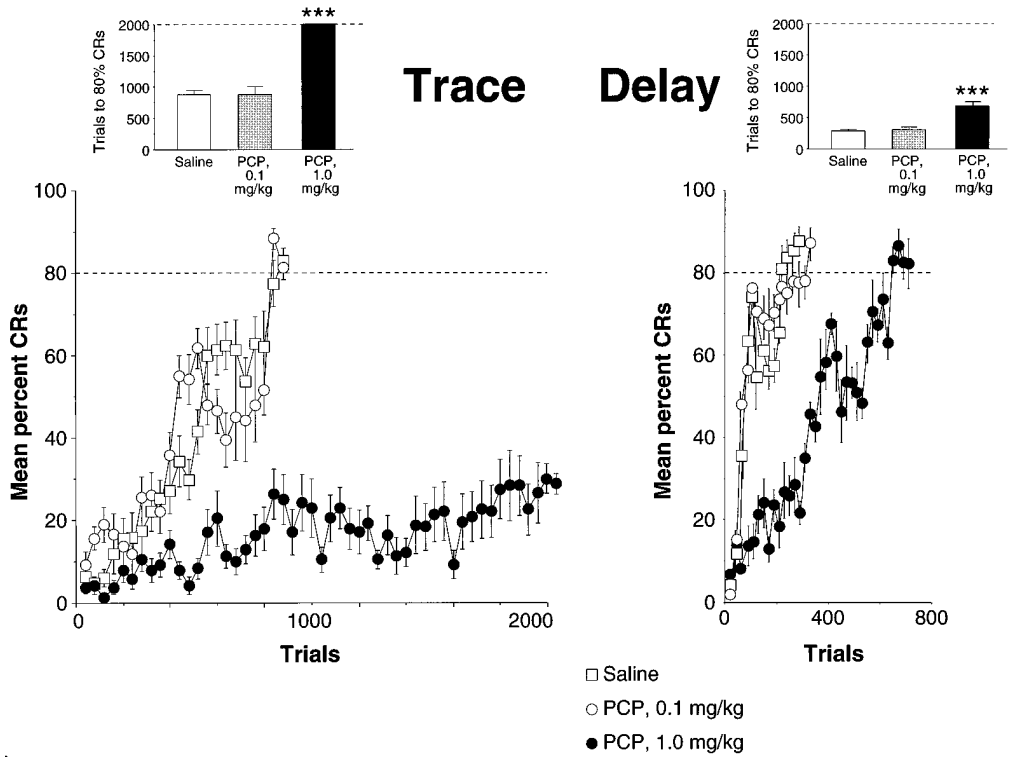

Fig. 2. The learning deficit produced by daily treatment with PCP was both dose- and task-dependent. Trace-conditioning (left panels) was more severely affected than delay-conditioning (right panels). Doses of 1.0 mg/kg of phencyclidine blocked acquisition of 500-msec trace eyeblink conditioning (left inset). No trace-conditioned rabbit treated with 1.0 mg/kg of PCP reached behavioral criterion (80% CRs), as seen in the learning curves (bottom left). Both the number of trace CRs per session and the rate of acquisition of these CRs was unaffected by a low dose of PCP (0.1 mg/kg). Doses of 1.0 mg/kg of PCP also significantly impaired delay eyeblink conditioning, but did not produce a complete block of learning (right inset). Instead, PCP-treated rabbits required slightly more than twice as many trials to reach criterion, with subsequent slowing of the learning curves (bottom right). Lower doses of PCP were without effect on delay-conditioning. [It should also be noted that the delay-conditioned task was much easier for rabbits to acquire than the trace-conditioned one, requiring considerable fewer trials to reach criterion (see also Thompson *et al.*, 1996a)]. The range of doses tested were without effect on the UR within the same trials in which these associative deficits were observed, or on nonspecific responding to tone CSs or to airpuff USs in pseudoconditioning (see table 2).

TABLE 3

Daily treatment with moderate doses of PCP (1.0 mg/kg) also had no effects on URs, although learning of both trace- and delay-conditioned eyeblinks was impaired at doses up to 1.0 mg/kg (i.m.).

Training	Dose of PCP	UR Amplitude (mV)	UR Peak Latency (msec)	CR Frequency (% Within ISI Used for Conditioning)
Trace-conditioned				
	0.0	3103 ± 167	197 ± 33	
	0.1	3016 ± 138	211 ± 44	
	1.0	3067 ± 153	209 ± 41	
Delay-conditioned				
	0.0	2956 ± 143	201 ± 33	
	0.1	3020 ± 151	213 ± 28	
	1.0	2922 ± 147	196 ± 25	
	2.0	2214 ± 133[b]	229 ± 32	
	5.0	1732 ± 169[c]	281 ± 37[b]	
	10.0	1157 ± 188[d]	328 ± 39[c]	
Pseudoconditioned				
	0.0	2984 ± 148	206 ± 29	13.1 ± 4.9
	1.0	3013 ± 153	220 ± 33	14.8 ± 5.4

[a] With high doses of PCP (≤2.0 mg/kg daily), significant dose-dependent anesthetic reductions in UR amplitudes were observed. Similar observations about a lack of effect on response sensitivity were obtained in pseudoconditioning control experiments with saline and PCP (1.0 mg/kg daily) treated rabbits.
[b] $P < .01$.
[c] $P < .004$.
[d] $P < .0001$.

occurred at all doses tested. These short-latency CRs typically ended hundreds of milliseconds before US onset (a nonadaptive shift).

Even at low doses (10 μg/kg daily) which had no effect on the rate of acquisition of the trace-conditioned CR (*i.e.,* eyeblinks within the trace interval), MK-801 shifted CR latencies to the early portion of the trace interval, rather than the typical late portion of the interval (see fig. 7). These leftward temporal shifts within the trace interval, even at low doses of MK-801, are similar to those observed in the same task after complete hippocampectomy (Moyer *et al.*, 1990). Moyer reported as a major effect that hippocampectomized rabbits exhibited a very small number of NMRs within the trace interval, too few to indicate significant acquisition across training. However, more than half of the few CRs that occurred were characterized as short-latency CRs (*i.e.,* with onset early in the trace interval). Daily treatment with MK-801 produced no significant variation in CR latency in the delay-conditioned task ($P > .2$). The mean CR latencies for MK-801-treated trace-conditioned rabbits were significantly different from those of controls [$F\ 4,25 = 10.3$, $P < .004$]. As with hippocampal lesions, the major dose-dependent effect noted for MK-801 was on the absolute number rather than on the latency of CRs, as increasingly fewer CRs of any latency (long or short) were observed with increased dosage. However, the leftward timing shift was apparent at the lowest

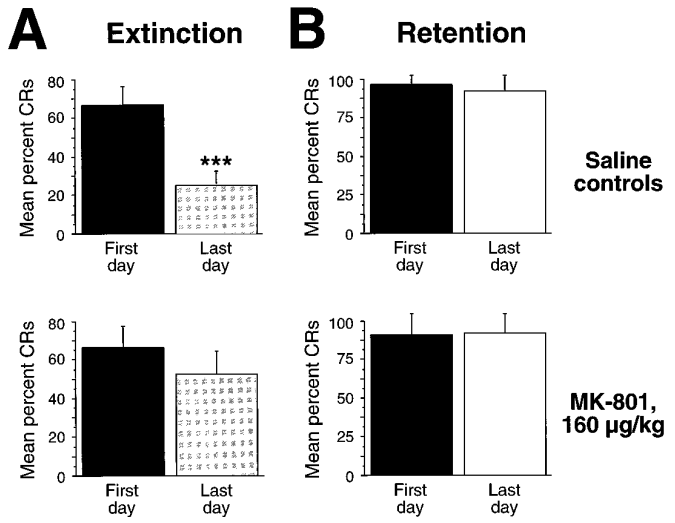

Fig. 3. MK-801 produced differential effects on postacquisition performance, depending not on the task learned but on the stimulus contingencies presented after learning had previously occurred. A, MK-801 severely impaired extinction of both trace- and delay-conditioned eyeblink responses. When the CS was presented alone (unpaired with the US), saline-treated controls extinguished rapidly, reaching low levels of CR performance within three daily sessions. Rabbits given 160 μg/kg of MK-801 daily, however, after previously acquiring the CR, exhibited little or no extinction, instead continuing to exhibit relatively high rates of responding to the unpaired CS. B, MK-801 treatment had no effects on retention of a previously learned CR. Both saline control rabbits and those receiving daily doses of 160 μg/kg of MK-801 continued to perform at rates meeting or exceeding behavioral criterion, although neither group had prior experience with MK-801 treatment. This is consistent with findings of the effects of MK-801 in other behavioral model systems.

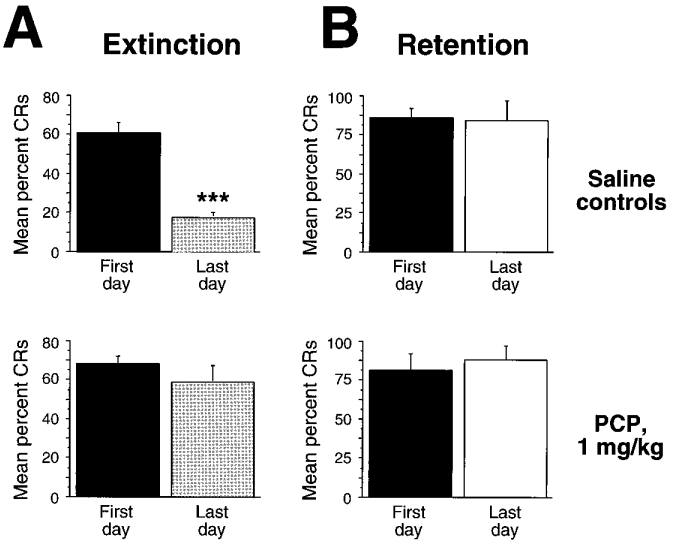

Fig. 4. Postacquisition PCP treatment severely impaired extinction of both trace- and delay-conditioned eyeblink responses, but had no effects on retention (performance of a previously learned response). No task-dependent differences in these effects were observed ($P > .4$), so our data combine results from both trace and delay conditioning. For both postacquisition tests, rabbits given daily saline vehicle injections were trained to a criterion of 80% CRs per session. For extinction, they were then were treated daily for three subsequent 80 trial unpaired CS sessions with either saline or 1.0 mg/kg of PCP (this dose of PCP produced significant learning deficits if treatment preceded training during acquisition; see fig. 1). Extinction was severely impaired, with CR performance remaining relatively stable even after repeated unpaired CS presentations. However, postacquisition PCP treatment no effect on retention of the previously acquired CR. For this testing, the CS was presented paired with the US for 3 subsequent 80 trial sessions, with rabbits receiving either saline or 1.0 mg/kg of PCP. Performance of the CR remained asymptotic at criterion levels across sessions with CS-US pairing. URs were unaffected by treatment with PCP in postacquisition testing (data not shown).

doses given (10 μg/kg), was also seen when the number of CRs performed was affected (at doses $\geq$ 40 μg/kg; see examples in figs. 6 and 7), and was present even when CR performance was almost fully abolished (at doses $\geq$ 80 μg/kg).

PCP also altered CR timing in acquisition. Treatment with PCP altered the timing of conditioned responses in acquisition of trace- but not delay-conditioning, resulting in performance of short-latency CRs. Figure 8 illustrates typical delay-conditioned CRs observed at criterion both for saline treated controls (top panel) and for subjects treated with 1.0 mg/kg of PCP (bottom panel). Figure 9 shows the distributions of CR latencies observed for trace-conditioned control rabbits and for those receiving PCP, and illustrates the observed differences between short- and long-latency CRs. Control rabbits exhibited typical eyeblink CRs, with CR onset timed so that the nictitating membrane remained extended at the onset of the airpuff US. The CR thus partly shielded the cornea from the aversive airpuff. Rabbits receiving PCP exhibited atypical short-latency CR distributions in trace-conditioning. Even low doses of PCP (0.1 mg/kg daily) greatly altered the timing of trace-conditioned eyeblink CRs, although no effect on CR numbers per se was noted at this dose (as described above; see fig. 2). The short-latency CRs exhibited by PCP-treated rabbits began very early in the trace interval, and ended too soon to shield the cornea from the aversive US. The mean CR latencies for PCP-treated rabbits were significantly different from those of control subjects in trace-conditioning [$F(2,15) = 16.7$, $P < .0002$], being shifted dramatically to the left in the CR latency plots (fig. 9). No leftward shift was observed in delay-conditioning ($P > .3$). These findings are consistent with data obtained in MK-801-treated rabbits trained in the same tasks.

Discussion

Our study demonstrates that in an animal model learning system daily treatment with noncompetitive NMDA antagonists produces profound learning impairments, with the severity and type of impairment varying with the dose used and the type of learning task observed. Our study is the first to directly compare MK-801 and PCP's effects on trace- and delay-conditioning of eyeblink responses, and to compare their effects on extinction and on retention of conditioned eyeblinks. The results indicate a critical and necessary involvement of NMDA receptors in hippocampus-dependent trace-conditioning, and a facilitatory involvement in cerebellar-dependent delay-conditioning. They also are consistent with the hypothesis that the learning deficits induced by PCP in eyeblink conditioning are a result of PCP's effects on NMDA receptors.

Previous work showed that moderate doses (50-100 μg/kg) of MK-801 dose-dependently slow acquisition of delay eyeblink conditioning in rabbits (Robinson, 1993), but are without effect on latent inhibition of the same task by CS preexposure (Robinson *et al.*, 1993). Additionally, ketamine has been shown to impair extinction after delay-conditioning

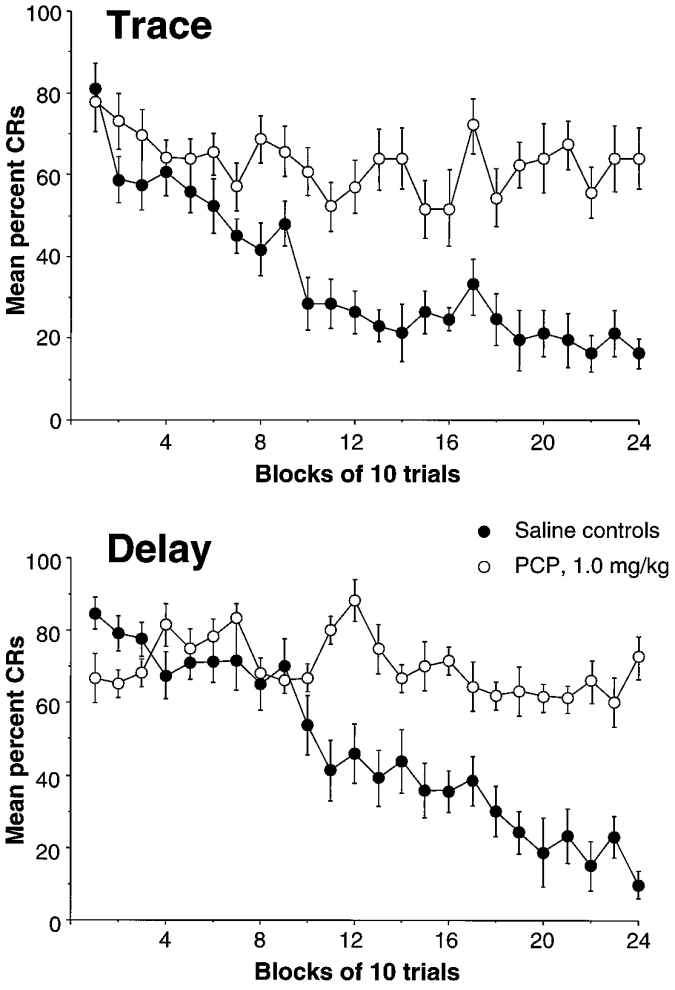

Fig. 5. Extinction curves for delay- (top panel) and trace-conditioned (bottom panel) rabbits show the severe impairment of extinction resulting from daily postacquisition treatment with 1.0 mg/kg of PCP. Saline-treated rabbits were first trained to a criterion of 80% CRs per session using paired CS-US trials, then given an additional three sessions of extinction using unpaired CS alone presentations. Rabbits treated postacquisition with PCP exhibited little or no extinction of the CR. Controls exhibited normal extinction curves, progressively performing fewer and fewer CRs with repeated unpaired CS presentations. No significant task-dependent differences were noted comparing extinction in the two eyeblink paradigms.

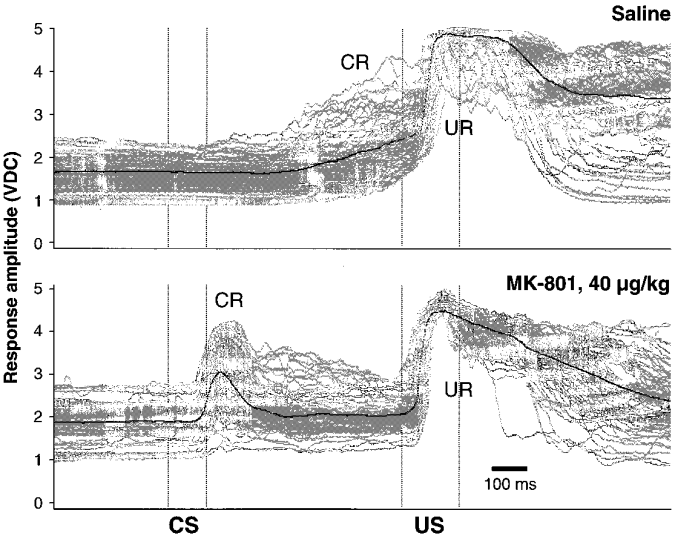

Fig. 6. MK-801 treatment produced aberrant CR timing in trace- but not in delay-conditioning, an effect reminiscent of the effects of hipocampal lesions. These timing abberations are illustrated in data from a set of sessions in which trace-conditioned rabbits reached behavioral criterion. In the top trace, individual trials (gray) and the averaged response for an 80 trial session (black) exhibit the long-latency CRs of a control rabbit. In the bottom trace, short-latency CRs produced by a rabbit treated with 40 μg/kg of MK-801 daily are shown. Rather than blunt US presentation to the cornea, as in controls, short-latency CRs typically ended long before US onset. No shift in CR latencies were observed in delay-conditioned rabbits treated with MK-801 (data not shown). Similar effects were also observed at a lower dose (10 μg/kg of MK-801), where acquisition rates were unaffected.

(Scavio *et al.*, 1992). Our study extends these findings, additionally showing that NMDA antagonists block acquisition of trace conditioning, alter trace CR timing and block extinction (but not retention) in both eyeblink tasks. The model learning system used has direct human parallels. Humans and rabbits, for example, exhibit similar impairments in acquisition of eyeblink conditioning as a function of aging (Solomon *et al.*, 1988; Thompson, 1988; Thompson *et al.*, 1996a). These parallels extend the relevance of our findings beyond the model system tested to a broader population. Phencyclidine is a cheap and commonly abused street drug, whose use by humans has frequently been associated with sociopathic behaviors and violence (Allen and Young, 1978; Nicholi, 1983; Pearlson, 1981; Showalter and Thornton, 1977). The impact of short- or long-term PCP intake on human learning and memory are relatively unknown. The demonstration of profound learning impairments in an animal model learning system after daily PCP use suggests that similar learning disturbances may occur in human users as well, and invites further study.

The data from our study are consistent with the hypothesis that the hippocampus may be a primary site of action for the observed learning deficits induced by the NMDA antagonist MK-801 or by PCP, although they certainly do not definitively address this question. Early observations of the psychotomimetic effects of high doses of PCP in humans (reviewed in Domino, 1964) led to studies suggesting that the drug might have primary actions on the limbic system, in particular on the hippocampus (Adey and Dunlop, 1960).

The cerebellar system, which is critically involved in other learning paradigms (Thompson, 1986, 1990), is also rich in NMDA receptors (Rosenmund *et al.*, 1992). The cerebellum and related circuitry are critical brain regions for all forms of eyeblink conditioning. Small lesions localized to the anterior interpositus nucleus eliminate the ability of rabbits to acquire short delay-conditioned eyeblink tasks on the side of the lesion, eliminate conditioned responses learned before the lesion (McCormick and Thompson, 1984) and block acquisition or expression of trace-conditioned responses (Woodruff-Pak *et al.*, 1985). These effects on learning of eyeblink CRs are seen in the absence of any effect on URs to an airpuff or shock US.

Clearly, different brain regions have different functional roles in learning, although certain of these functions may demonstrate considerable overlap. Eyeblink conditioning (as well as other forms of learning) is almost certainly a distributed function within the brain (see Thompson, 1991; Squire, 1987). Although the cerebellar circuitry is necessary for

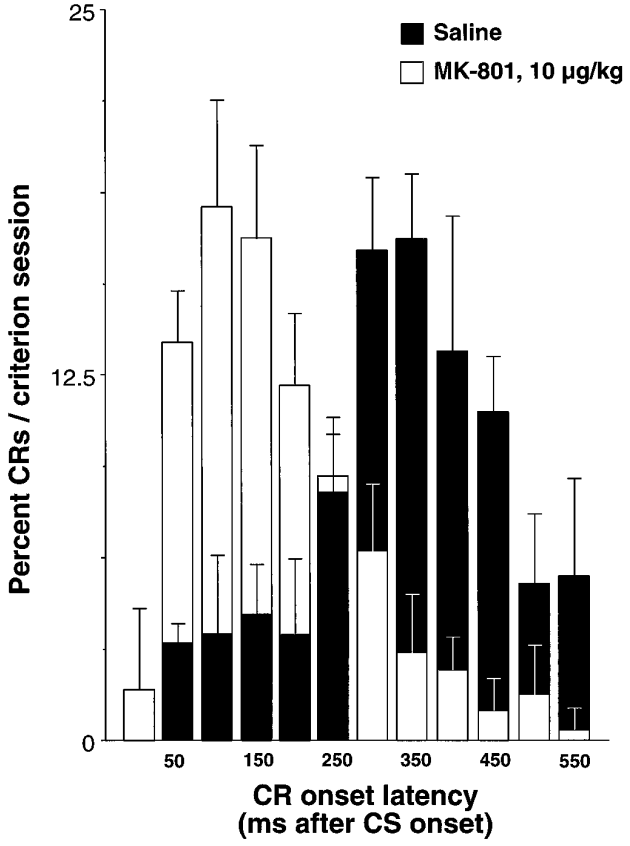

Fig. 7. Daily MK-801 treatment severely altered the distribution of trace-conditioned CR onset latencies. Saline-treated control animals exhibited normal CR latency distributions over the course of training. By the time criterion was reached, a reliable and tightly clustered distribution of CR onset latencies was seen, with onset of the CR clustering at intervals shortly before presentation of the US. The number of short-latency CRs decreased with training (data not shown), although the number of long-latency CRs increased quite dramatically across trials. MK-801-treated subjects learned aberrantly timed short-latency CRs, with onsets shortly after CS onset, and frequently occurring during CS presentation. This was seen even in data from rabbits receiving fairly low doses of MK-801 (10 μg/kg daily). More than 80% of the CRs at low doses were short-latency, *i.e.,* they began early in the trace ISI (or even during CS presentation) and ended prior to airpuff US onset. The number of short-latency, rather than long-latency, CRs increased across trials in this group.

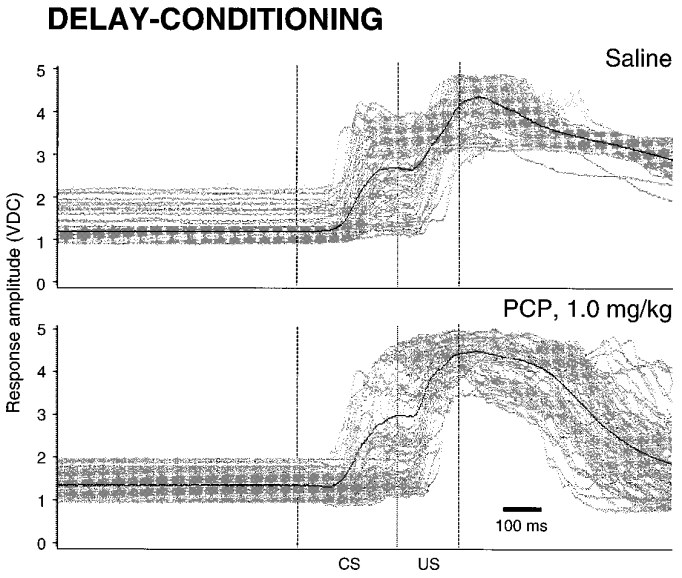

Fig. 8. PCP treatment had no effect on conditioned response timing in delay-conditioning. Trial-by-trial data (gray traces) and averaged session data (black traces) are shown for two rabbits for the session in which they reached behavioral criterion. CR onset or peak latencies, UR amplitudes and UR peak latencies were all unaffected by treatment even with a moderate dose (1.0 mg/kg) of PCP, although this same dose effectively slowed acquisition (fig. 1) and blocked extinction (fig. 2) of delay-conditioning.

learning of both trace- and delay-eyeblink responses, the hippocampus may play an important role even in delay-conditioned tasks that are not usually defined as hippocampally dependent (Solomon *et al.*, 1983; Akase *et al.*, 1989). The number of binding sites for noncompetitive NMDA antagonists, however, is significantly fewer in the cerebellar than in the hippocampal region and the binding affinity is significantly different (Ebert *et al.*, 1991). The distribution of subclones of the NMDA receptor is also different in the two regions (Monyer *et al.*, 1994). Taken together with our data, these suggest that NMDA receptor function may be quite different in hippocampal and cerebellar regions. The hippocampally dependent nature of the trace-conditioning task used in our study (Moyer *et al.*, 1990) and the ability of MK-801, PCP and of antagonists of other major hippocampal neurotransmitter systems (*i.e.,* cholinergic antagonists, *re.* Solomon *et al.*, 1983) to also disrupt delay-conditioning suggest that the hippocampus plays an important (if not always critically required) role in both variants of eyeblink conditioning. The short-latency CRs in trace- but not delay-conditioning reported here and after hippocampal lesions (Moyer *et al.*, 1990) are somewhat similar to the CR timing disruptions observed in delay-conditioning after cerebellar cortex lesions (Perrett *et al.*, 1993), although URs were also altered by the cerebellar lesions but not by MK-801 or PCP (at doses that completely blocked CR acquisition) or by hippocampal lesions. These findings suggest further study is needed to resolve issues related to CR timing.

A cautionary note regarding the interpretation of our data is appropriate regarding the apparent differences in susceptibility of trace- and delay-conditioned tasks to NMDA receptor blockade. As noted, two specific eyeblink tasks were chosen that also demonstrate differing susceptibility to the effects of hippocampal ablation (see Akase *et al.*, 1989; Moyer *et al.*, 1990). However, not only do these tasks differ in CS-US overlap (*i.e.,* significant overlap in delay, no overlap in trace), but also in CS-US timing (the ISI). The major reason two paradigms using identical ISIs were not used was that no data comparing effects of hippocampal ablation in such paradigms are available; such studies are beyond the scope of the questions addressed. Instead, we chose two well-validated eyeblink paradigms, with acquisition in one blocked by hippocampal lesions (trace 500; Moyer *et al.*, 1990; Solomon *et al.*, 1986) and in the other unaffected (delay 250; Akase *et al.*, 1990; Schmaltz and Theios, 1972; Solomon and Moore, 1975) to represent the two major pardigms. As noted in "Methods," data from our own laboratory (Thompson *et al.*, 1996a) and others (Solomon *et al.*, 1996) have shown that, in the trace task used here (with a 600-msec ISI and a 500-msec trace interval) and in similar trace tasks, young rabbits require significantly more training to reach criterion than in delay tasks using identical ISIs. Further, no differences in

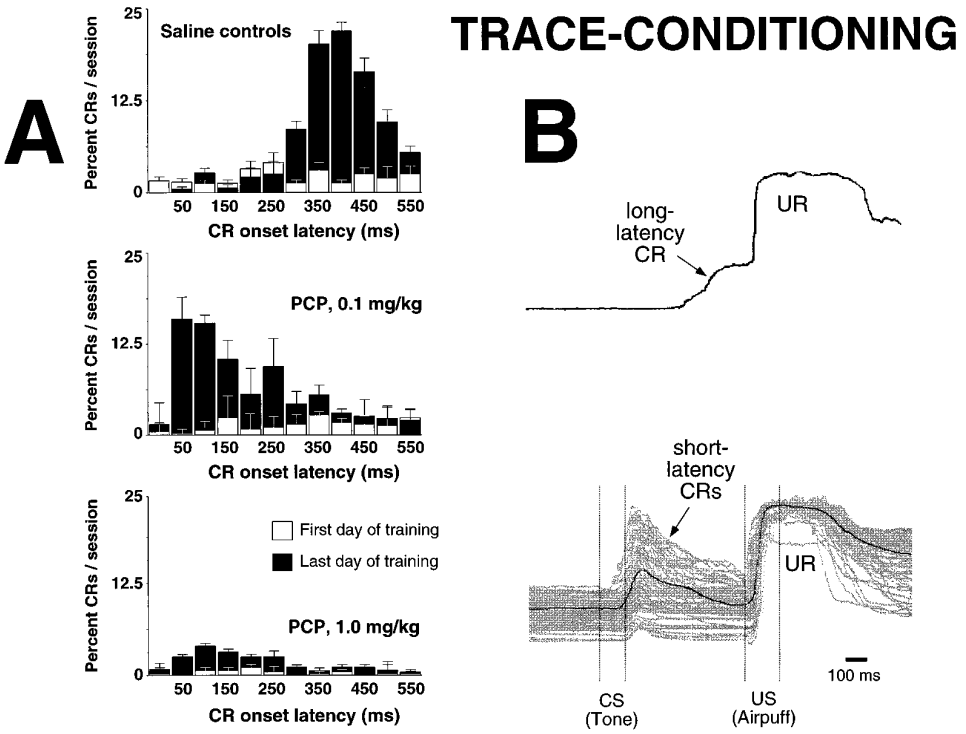

Fig. 9. Daily PCP treatment did severely alter conditioned response timing in trace-conditioning. A, CR latency distributions were strongly shifted by daily treatment with PCP, even low doses (0.1 mg/kg). Saline-treated control animals exhibited normal timing of CRs over the course of training. An initial random distribution of a small number of CRs with varying onset latencies (after CS onset but prior to US onset) changed, over the course of training, to a new learned configuration. At the end of training, a reliable and tightly clustered distribution of CR onset latencies was seen, with onset of the CR shortly before presentation of the US. The number of short-latency CRs decreased with training, although the number of long-latency CRs increased quite dramatically across trials. PCP-treated subjects learned aberrantly timed short-latency eyeblinks, with CR onsets shortly after CS presentation. This was seen even in data from rabbits receiving low doses of PCP (0.1 mg/kg daily). Although this low dose did not affect the increase in CR numbers operationally defined as acquisition of the CR (see fig. 1), it did severely impair acquisition of long-latency CRs. More than 60% of the eyeblinks of low dose (0.1 mg/kg) PCP-treated rabbits were short-latency, *i.e.,* they began early in the trace interval and ended before airpuff US onset. The number of short-latency, rather than long-latency, CRs increased across trials in this group. Rabbits receiving moderate doses of PCP (1.0 mg/kg daily) showed many fewer CRs, but with a similar short-latency timing distribution. B, Top, long-latency eyeblink CR exhibited by a control rabbit, timed such that the nictitating membrane was extended at the onset of the airpuff US. Bottom, PCP-treated rabbits, even those receiving low doses (0.1 mg/kg daily), exhibited short-latency CRs. These responses were initiated shortly after CS onset and frequently ended well before US onset. These short-latency responses by PCP treated animals strongly resembled the short-latency CRs observed after partial or total hippocampal ablation (Solomon *et al.*, 1986; Moyer *et al.*, 1990). Functionally, PCP thus produced deficits in CR timing similar to those observed after hippocampal lesions.

trials to criterion were observed in delay-conditioning comparing short with long ISIs, ranging from 250 to 900 msec (Thompson *et al.*, 1996a; Solomon *et al.*, 1996), including comparisons of the specific ISIs used. Thus, although it is possible that delay-conditioning at longer ISIs would be more susceptible to the effects of NMDA receptor blockade (or of hippocampal lesions) than is apparent, currently available data argue against such an effect.

The requirement for NMDA receptor activation appears to be absolute in the trace-conditioned task used here (as trace-conditioning could be blocked with moderately high doses of MK-801 or of PCP), but facilitatory in the delay-conditioning task used (with slowing but not blocking of acquisition by the same doses MK-801 or PCP). Extinction of the conditioned eyeblink also appears to require NMDA receptor activation, although retention of the learned response does not. NMDA receptor function appears to be required for acquiring many forms of learning (Morris, 1989). Specifically, studies with short-term MK-801 treatment (Robinson *et al.*, 1989; Whishaw and Auer, 1989) have shown dose-dependent impairments of acquisition in spatial tasks that are also severely impacted by hippocampal damage. Retention or performance of previously learned spatial tasks, however, is unaffected by MK-801 (Heale and Harley, 1990; McLamb *et al.*, 1990; Shapiro and Caramanos, 1990). The data presented are consistent with these findings. Conversely, studies from our laboratory (Thompson *et al.*, 1992) and others (Monahan *et al.*, 1989; Herberg and Rose, 1990; Schwartz *et al.*, 1991) have shown that agonists of the strychnine-insensitive glycine B coagonist site on the NMDA receptor/channel-complex have a complementary effect, enhancing acquisition in a number of learning tasks, including trace eyeblink conditioning.

As noted, the effects of daily MK-801 or PCP treatment in our study in several ways mimic the effects of hippocampal lesions on trace eyeblink conditioning (Moyer *et al.*, 1990; Solomon *et al.*, 1986) and on other tasks (Robinson *et al.*, 1989), interfering with several significant aspects of normal adaptive conditioning. Our findings appear to be consistent with recent reports that hippocampal lesions made before or shortly after training prevent acquisition of the same 500-msec trace-conditioned eyeblink task used, but that lesions made some time after acquisition do not affect the previously learned response (Kim *et al.*, 1995). Hippocampal lesions have also been reported to block extinction of the eyeblink

CR, even in delay-conditioning, acquisition of which is unaffected by hippocampal lesions (Akase *et al.*, 1989).

It is notable that MK-801 or PCP affected acquisition and extinction in both trace- and delay-conditioning across a range of doses, but produced a novel dissociation between response timing effects and response acquisition effects at the lower doses tested only in trace-conditioning. Numerous studies have supported the hypothesis that the hippocampus plays a critical role in the short-term information processing normally underlying the formation of associative memories (Rawlins, 1985; Olton, 1983). Solomon (1979) suggested that the hippocampus plays a major role in the timing relationships associating sensory stimuli with behavioral responses in eyeblink conditioning as well as other learning tasks. In a blocking paradigm using delay eyeblink conditioning, for example, Solomon (1977) demonstrated that hippocampally ablated rabbits failed to discriminate between stimuli that were not associatively paired with a US and those that were, exhibiting equal numbers of conditioned responses to both paired and unpaired stimuli. The similarity in the short latency CRs observed in trace-conditioned rabbits after partial (Solomon *et al.*, 1986) or complete (Moyer *et al.*, 1990) hippocampal ablations and in our study as a result of daily MK-801 treatment, even at fairly low and otherwise behaviorally ineffective doses, suggest that hippocampal NMDA receptor function is required for conditioning of normally timed adaptive eyeblink CRs. The lack of effect on response timing and the slowing but not blocking of acquisition in delay-conditioning suggests a less active role for NMDA receptors in delay-conditioning. The effects on extinction in both tasks suggest that extinction uses somewhat different neural subsystems than those required for acquisition of eyeblink conditioning. They are consistent with earlier hypotheses that the hippocampus contributes strongly to extinction of learned responses, perhaps by mediation of behavioral inhibition (Douglas, 1967).

Some of the ataxic and stereotyped behaviors produced in earlier animal studies with high doses of phencyclidine (*e.g.,* Contreras, 1990) are reduced after prolonged daily treatment with high doses of PCP (Leccese *et al.*, 1986). This reduction has been interpreted as development of behavioral, if not pharmacological, tolerance to the drug. In our studies, however, no behavioral tolerance of the effects of PCP (nor of MK-801) were observed, even after prolonged treatment (25 days). It remains to be seen whether chronic treatment with phencyclidine or other NMDA antagonists result in long-term deficits in learning ability, once treatment ceases. Both single and repeated infusions (four daily doses) of PCP or MK-801; however, have been associated with pathological changes in retrosplenial and cingulate cortical neurons (Olney *et al.*, 1989) that may also produce deficits in learning and memory, and provide clues for possible treatments of severe neuropsychiatric syndromes such as schizophrenia (Olney, 1990; Javitt and Zukin, 1991).

Phencyclidine's interactions as an NMDA receptor ligand have been extensively studied. By the late 1970's, although "PCP receptors" with submicromolar affinity for PCP had been identified in brain tissue (Zukin and Zukin, 1979; Vincent *et al.*, 1979), their association with the NMDA receptor/channel-complex had not yet been elucidated (Johnson *et al.*, 1988). Sigma opiate receptors (to which PCP binds with less affinity) and "PCP receptors" have subsequently been differentiated pharmacologically (Gundlach *et al.*, 1985; Majewska *et al.*, 1989). Similarly, PCP *in vitro* interacts with low affinity with aminergic and cholinergic receptors and has direct effects on potassium and sodium channels, but at doses far exceeding those required for blockade of NMDA-mediated neurotransmission (Domino, 1986; ffrench-Mullen and Rogawski, 1989; Javitt and Zukin, 1991). PCP dose- and use-dependently blocks the ionophore of the NMDA receptor (ffrench-Mullen and Rogawski, 1992), in a manner comparable to that of other noncompetitive antagonists of the NMDA receptor. It is most likely that the dose-dependent behavioral effects of PCP reported, which mirror those obtained with MK-801, were mediated via noncompetitive blockade of the NMDA receptor's ionophore. Similar deleterious effects on learning have been obtained in other hippocampally dependent paradigms using MK-801 (McLamb *et al.*, 1990; Ward *et al.*, 1990), which has almost no affinity for sigma opiate receptors and very high affinity for the ionophore binding site (Wong *et al.*, 1988; Reynolds *et al.*, 1987). The anesthetic effects observed at higher doses, however, may involve interactions with other proteins (see Allaoua and Chicheportiche, 1989).

NMDA receptor activation appears to be critically involved in many forms of neural plasticity, not solely limited to learning nor to (some forms of) LTP. NMDA receptors play important facilitatory roles in neuronal development in many brain regions (Kleinschmidt *et al.*, 1987). NMDA receptors have also been strongly implicated in neurodegenerative processes associated with aging (Procter *et al.*, 1989; Jansen *et al.*, 1990), with seizure disorders (Dingledine *et al.*, 1990; Singh *et al.*, 1990), and with the excitotoxic sequelae of events after ischemic insult (Himori *et al.*, 1990; McIntosh *et al.*, 1990).

Noncompetitive use-dependent blockade of the cation channel of the NMDA receptor/channel-complex with MK-801, even at low doses, degraded response timing in trace- but not delay-conditioning, resulting in acquisition of aberrantly timed CRs. At intermediate doses, MK-801 slowed acquisition in both tasks significantly. At still higher doses, MK-801 still further slowed acquisition of delay-conditioning, and completely blocked acquisition of the trace-conditioned eyeblink. Still higher doses exhibited nonassociative effects on the UR, probably due to anesthetic effects on sensory processing. Parallel experiments in our laboratory have shown that agonists rather than antagonists of the NMDA receptor have opposite effects from those reported (see Thompson *et al.*, 1992), enhancing acquisition rates of eyeblink conditioning. Our experiments thus complement our own and others' data, and further support a necessary role for NMDA receptors in trace-eyeblink conditioning, and a facilitatory role in delay-conditioning. NMDA receptors are highly enriched in specific dendritic regions of the hippocampus, and MK-801 or PCP's blockade of these receptors results in learning deficits similar to those seen after total hippocampal ablations (Moyer *et al.*, 1990). NMDA receptors also appear to play a facilitatory role in delay-conditioning, speeding acquisition when functional, but without being absolutely required for learning to occur. The effects observed indicate an active functional role for NMDA receptors in the processes required for learning a new conditioned eyeblink response, for extinguishing that learning, but not for long-term memory of the learned response.

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Send reprint requests to: Dr. L. T. Thompson, Department of CM Biology, Northwestern University Medical School, 303 E. Chicago Ave., Chicago, IL 60611-3008.