

N-Methyl-D-Aspartate Receptor Antagonist APV Blocks Acquisition but Not Expression of Fear Conditioning

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The role of *N*-methyl-D-aspartate (NMDA) receptors in Pavlovian fear conditioning was examined using the NMDA antagonist DL-2-amino-5-phosphonovaleric acid (APV). Either APV (5 μ g/rat) or saline was administered before the training phase, the testing phase, or both. APV completely blocked acquisition but not expression of fear conditioning. The L enantiomer of APV did not affect the acquisition of conditional fear. To separate encoding from consolidation processes, APV was administered either before or immediately after the footshock unconditional stimulus (US) during the training phase. The results indicate that APV must be present during the US to produce its effects on fear conditioning. The behavioral effect of the drug is not due to analgesic action because APV did not alter pain sensitivity. The data suggest that NMDA receptors are critical for the acquisition but not expression of fear conditioning. These effects on fear conditioning are parallel to the *in vitro* effects of APV on the acquisition but not expression of long-term potentiation (LTP) and suggest that endogenously generated NMDA-dependent LTP participates in the neural plasticity underlying fear conditioning.

Fear conditioning is a rapidly acquired and persistent form of simple associative learning. When a rat is placed in a novel chamber and presented with a mild footshock, shortly afterward the animal freezes. For rats, this freezing response is the dominant species-specific defense reaction to conditional fear (Fanselow, 1986a). It is characterized by an immobile, crouching posture and has proved to be a reliable index of fear (R. J. Blanchard & D. C. Blanchard, 1969; Fanselow, 1980). Upon return to the chamber, either 24 or 48 hr later, the animal again freezes (Fanselow, 1980, 1982). The freezing response represents a form of Pavlovian conditioning because it is entirely caused by an association between contextual cues of chamber and shock (Fanselow, 1980, 1982, 1986a). In other words, it is a conditional response to conditional stimuli (CS) that are associated with the footshock unconditional stimulus (US; R. J. Blanchard & D. C. Blanchard, 1969; Bolles & Collier, 1976; Fanselow, 1980).

Because fear conditioning is a potent form of learning, it must result in some immediate but permanent change in the animal's central nervous system. However, the nature of this physical change is unknown. Long-term potentiation (LTP) of excitatory synaptic transmission appears to be a good candidate for such a fear memory mechanism. LTP is a rapidly acquired, stable enhancement in synaptic efficacy that

follows a brief high-frequency stimulation of a neural pathway (Bliss & Gardner-Medwin, 1973; Bliss & Lomo, 1973). Because of the rapidity with which it can be established and because of its longevity, LTP has been proposed as a physiological mechanism for learning and memory (Bliss & Lomo, 1973; Lynch & Baudry, 1984).

N-methyl-D-aspartate (NMDA) receptors appear to play an important role in the development of LTP (Collingridge, 1985; Collingridge, Kehl, & McLennan, 1983; Muller, Joly, & Lynch, 1988). The NMDA receptor is one of five specific classes of excitatory amino acid (EAA) receptors (Monaghan, Bridges, & Cotman, 1989). NMDA antagonists have been shown to block induction of LTP (Collingridge et al., 1983; Harris, Ganong, & Cotman, 1984; Muller et al., 1988). However, they do not affect expression of already-established LTP nor do they impair ordinary synaptic transmission (Collingridge et al., 1983; Harris et al., 1984; Muller et al., 1988). Therefore, the potentiated synaptic transmission appears to be expressed by non-NMDA EAA (e.g., quisqualate-kainate) receptors, whereas the NMDA synaptic component remains unchanged (Davies, Lester, Reymann, & Collingridge, 1989; Muller et al., 1988).

Several studies have used NMDA antagonists in an attempt to establish a link between LTP and learning (Morris, Anderson, Lynch, & Baudry, 1986; Staubli, Thibault, Di-Lorenzo, & Lynch, 1989; Tonkiss, Morris, & Rawling, 1988). For example, chronic intraventricular infusion of DL-2-amino-5-phosphonovaleric acid (APV) has been reported to impair spatial learning in rats, although spatial memory and visual discrimination learning remained intact (Morris et al., 1986). NMDA receptors also appear to play a role in olfactory discrimination learning because APV impaired performance in an olfactory discrimination task as well (Staubli et al., 1989). Although these studies implicate NMDA receptors in learning, they did not allow for a clear separation of APV effects on learning as opposed to performance (Morris et al.,

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1986; Tonkiss et al., 1988) or did not control for the possibility that performance impairments were caused by state-dependent generalization decrement (Staubli et al., 1989).

The present series of experiments examined the role of NMDA receptors in Pavlovian fear conditioning by assessing the effects of APV on both acquisition and performance of conditional freezing. Because fear conditioning proceeds extremely rapidly (occurs even with a single CS-US pairing), we feel that fear conditioning represents an ideal place to look for NMDA effects on learning. In addition, procedures using freezing have been worked out that allow a clear separation of learning from performance and state-dependent effects (e.g., Helmstetter & Fanselow, 1987).

Experiment 1

To determine whether APV affects fear conditioning in a manner parallel to its *in vitro* effects on LTP, we used a 2×2 factorial design in which either APV or saline was administered before the training phase, the testing phase, or both. In this procedure, an effect of drug during training indicates a learning effect, whereas a drug effect during testing suggests an influence on performance. State dependent effects are indicated by a Training \times Testing interaction.

Method

Subjects and surgery. Forty-six experimentally naive adult female rats of Long-Evans descent, that weighed between 200 and 268 g, served as subjects. They were maintained in the University of California, Los Angeles, psychology department colony room with a 12:12-hr light-dark cycle. Rats were individually housed in hanging stainless steel cages with ad lib access to food and water.

Subjects were anesthetized with 45 mg/kg sodium pentobarbital and treated with atropine (0.3-ml injection volume at a concentration of 0.4 mg/ml). With the skull leveled between lambda and bregma, a 22-gauge stainless steel guide cannula (Plastic Products, Roanoke, VA) was aimed and implanted into the right lateral ventricle (coordinates: 0.5 mm posterior to bregma, 1.5 mm lateral to midline, and 3.2 mm ventral to the surface of the cortex). All rats were given a minimum of 7 days to recover after surgery. During this time subjects were handled daily, and each dummy cannula was removed and replaced with a clean one.

Drugs and injection. APV (Sigma Chemical Co., St. Louis, MO) was dissolved in sterile (0.9% NaCl) saline. The intracerebroventricular (icv) infusions consisted of backloading the drug up a 28-gauge internal cannula (Plastic Products, Roanoke, VA) into polyethylene (PE-50) tubing (Intramedic No. 7411). The internal cannula extended 0.5 mm beyond the guide cannula. A 6- μ l injection volume was delivered with a 100- μ l Hamilton syringe at a rate of 6 μ l/min. The internal cannula remained in place for at least 1 min after the icv infusions.

Apparatus. Both training and testing took place in four identical observation chambers (28 \times 21 \times 10.5 cm; Lafayette Instrument Co., North Lafayette, IN) that were placed inside of sound- and light-attenuating chests. A white light bulb (1820 bayonet bulb, 28 V) that illuminated the chamber allowed the experimenter to observe and videotape the subjects' behavior through a double-paned, 25 \times 53 cm, clear plastic window in the front wall of each chest. Ventilation fans supplied background noise (78 dB, A scale).

The floor of each chamber was composed of 18 stainless steel rods (4-mm diameter), spaced 1.5 cm center to center, and wired to a

shock generator and scrambler (Lafayette Instrument Co., North Lafayette, IN). Each chamber was cleaned with an ammonium hydroxide (5%) solution before and after each subject.

Procedure. Subjects were randomly assigned to each cell of a 2×2 factorial design. The factors were APV or saline (SAL) infusion before the training and APV or saline infusion before the testing; group designations (training-testing treatments) were APV-APV ($n = 12$), APV-SAL ($n = 11$), SAL-APV ($n = 12$), and SAL-SAL ($n = 11$).

On Day 1, subjects were infused with either APV (5 μ g/rat) or saline and placed in the observation chambers. After 3 min, animals received three shocks (1 mA, 0.75-s duration, 20 s apart) and were then returned to their home cage 20 s after the last shock. Behavior was videotaped throughout the session.

Day 2 was the test day. Each rat was infused with either APV or saline and placed for 8 min in the chamber in which it had received shock the day before. Shocks were not presented during this test. Four animals were observed simultaneously, 1 in each of the four observation chambers.

During this phase, a time-sampling procedure was used to assess fear conditioned to the chamber. Every 2 s an observation of 1 of the 4 animals was made. Each animal was therefore scored once every 8 s. Behavior was judged to be freezing or not at the instant the sample was taken. *Freezing* was defined as the absence of any visible movement of the body and vibrissae except for movement necessitated by respiration. In all of the experiments reported in this article, the data were collected by a trained observer who was uninformed to the subject's treatment.

Histology. The subjects were overdosed with sodium pentobarbital, infused intraventricularly with 6 μ l India ink, and perfused intracardially with 0.9% saline, which was followed by 10% formalin. The brains were removed, and coronal sections were made along the cannula tract. Positive cannula placement was verified for each rat by the presence of ink in the ventricles. Data obtained from subjects with negative cannula placement were excluded from the statistical analyses. In Experiment 1, 7 rats were excluded from the analyses due to negative cannula placement.

In all of the experiments reported in this article, the cannula placements were verified by an experimenter who was uninformed to the subjects' treatment.

Results and Discussion

Figure 1 shows the mean freezing level of the four groups during the 8 min of the testing that occurred 24 hr after shock. Inspection of the figure indicates that APV given before the training phase markedly attenuated acquisition of conditional fear. This was supported by a two-way analysis of variance (ANOVA), which indicated a significant main effect of training treatment, $F(1, 38) = 24.76, p < .001$, but not testing treatment, $F(1, 38) = 1.39, p > .2$. The Training \times Testing interaction did not approach statistical reliability, $F(1, 38) < 1$. Thus, the effect of APV on acquisition cannot be attributed to either a performance deficit or state-dependent generalization decrement. Analogous to its effect on *in vitro* LTP studies (Collingridge et al., 1983; Muller et al., 1988), APV interferes with acquisition but not expression of conditional fear.

To determine whether APV had any effects on preshock or immediate postshock freezing behavior, we measured preshock baseline and postshock freezing responses every 1 s for all groups for 1 min before the first shock and during the three 20-s periods between shocks (Figure 2). None of

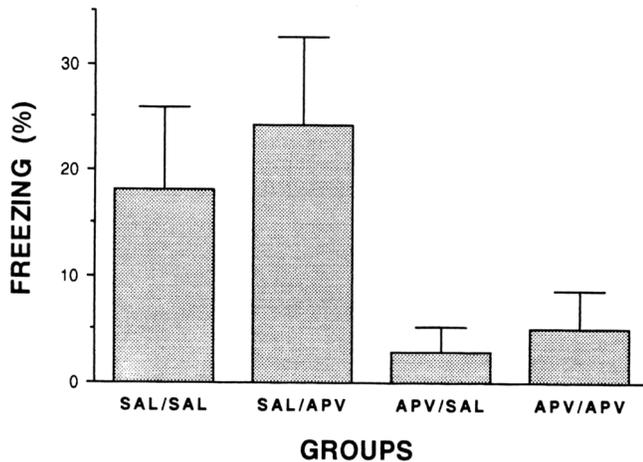


Figure 1. Mean (\pm SE) freezing of SAL/SAL ($n = 9$), SAL/APV ($n = 10$), APV/SAL ($n = 10$), and APV/APV ($n = 10$) groups 24 hr after three shocks expressed as the percentage of total observations during the 8-min test.

the rats froze before shock. This suggests that APV per se does not induce immobility in untrained animals. Animals pretreated with APV showed a freezing response during the postshock period equivalent to that of SAL pretreated rats; that is, APV had no effect on immediate postshock freezing, $F(1, 38) < 1$. Thus, although APV blocked learning of conditional fear, it had no effect on performance of fear during conditioning.

Experiment 2

A considerable amount of evidence indicates that the activity of racemic APV at NMDA receptors resides principally in the D enantiomer of this compound (Davies & Watkins, 1982; Evans, Francis, Jones, Smith, & Watkins, 1982; Watkins & Olverman, 1987). For example, icv administration of racemic APV, but not the inactive L enantiomer of APV, blocked induction of LTP in the dentate gyrus and impaired learning of a spatial task (Morris et al., 1986). In this second experiment, the L enantiomer of APV (L-APV) was included to demonstrate stereospecificity of the effect of APV on fear conditioning.

Method

Subjects and surgery. Thirty-three experimentally naive adult female rats (194–304 g) of Long-Evans descent served as subjects. Animals were implanted with icv cannulae and housed in a manner identical to those in Experiment 1.

Drug and injection. Both APV (racemic) and L-APV (Sigma Chemical Co., St. Louis, MO) were dissolved in sterile (0.9% NaCl) saline. Animals received either APV (5 μ g/rat), L-APV (2.5 μ g/rat), or saline in the same manner as described in Experiment 1. Based on Morris et al. (1986), we used the dose of L-APV that was half of our racemic APV dose. A 3- μ l injection volume was delivered at a rate of 3 μ l/min.

Apparatus and procedure. The apparatus that was used in Experiment 1 was also used in this experiment.

An equal number of subjects were randomly assigned to the following three groups: SAL, APV, and L-APV.

On Day 1, animals were infused with either APV, L-APV, or saline and placed in the observation chambers. After 3 min, animals received three shocks (1 mA, 0.75-s duration, 20 s apart) and were then returned to their home cage 20 s after the last shock.

On the next test day, each rat was placed back in the chamber for 8 min in the absence of shock. The amount of fear conditioned to the chamber was assessed in the same manner as previously described in Experiment 1.

Histology. The procedure was the same as that of Experiment 1. In this experiment, 2 rats were excluded from the analyses due to negative cannula placement.

Results and Discussion

Figure 3 depicts the mean freezing level of SAL, L-APV, and APV subjects during the 8 min of the testing that occurred 24 hr after the training phase. A one-way ANOVA indicated that the APV group showed a significant reduction in freezing compared with both SAL- and L-APV-treated animals, $F(2, 28) = 7.05$, both $p < .01$, Newman-Keuls. There was no difference in levels of freezing between SAL and L-APV groups. Thus, the behavioral effect of racemic APV appears to reside principally in the D enantiomer of the compound. This stereospecificity is consistent with an action at NMDA receptors.

Experiment 3

Immediate posttraining pharmacological manipulations often affect later performance on a variety of tasks, suggesting an influence on memory consolidation processes (Gallagher & Kapp, 1978; Izquierdo, Paiva, & Elisabetsky, 1980; Messing et al., 1979). Therefore, this third experiment was designed to compare the effects on fear conditioning of administration of APV just before shock exposure with the effects of APV administration immediately after shock exposure to

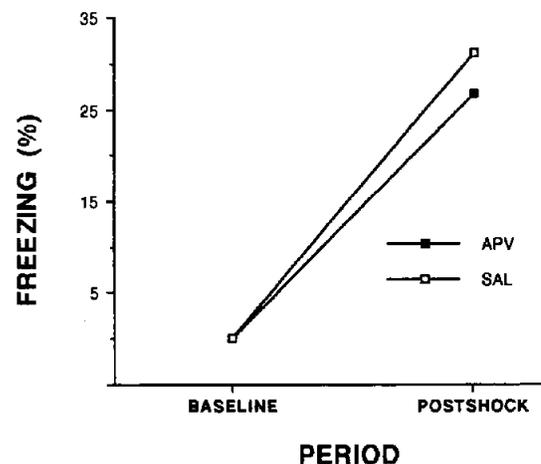


Figure 2. Mean percentage freezing of SAL ($n = 19$) and APV ($n = 20$) pretreated animals during 1 min before the first shock (baseline) and during the three 20-s postshock intervals. (Error bars were not included because they overlapped at all points.)

determine whether APV affects encoding or consolidation. To allow for a clearer separation of the encoding from consolidation periods, a one-shock rather than the three-shock, training procedure was used.

Method

Subjects. Thirty experimentally naive adult female rats (208–274 g) of Long-Evans descent were housed in a manner identical to those in Experiment 1.

Surgery, drug, and injection. The surgical procedure was identical to that of Experiment 1. Animals received either APV (5 μ g/rat) or saline in the same manner as described earlier. A 3- μ l injection volume was infused intraventricularly at a rate of 3 μ l/min. The infusion volume was cut in half from that of Experiment 1 because the design of this experiment required two injections within a brief period of time.

Apparatus and procedure. The apparatus used in this experiment was the same as that described for Experiment 1.

An equal number of subjects were randomly assigned to the following three groups: APV–SAL, SAL–APV, and SAL–SAL (pre-shock–postshock treatments).

On Day 1, subjects were infused with either APV or saline and placed in the observation chambers. After 3 min, animals received a single shock (1 mA, 1.5-s duration). They were removed from the chamber immediately after shock and infused with either APV or saline, and were then returned to their home cage.

On the next test day, each rat was placed back in the chamber for 8 min in the absence of shock. The amount of fear conditioned to the chamber was assessed in the manner already described.

Histology. This procedure was the same as that described earlier. In Experiment 3, 5 rats were excluded from the analyses due to negative cannula verification.

Results and Discussion

Figure 4 presents the mean freezing level of APV–SAL, SAL–APV, and SAL–SAL groups during the 8 min of testing that occurred 24 hr postshock. As can be seen in the figure, APV administration before the shock completely blocked freezing, whereas the immediate postshock APV injection

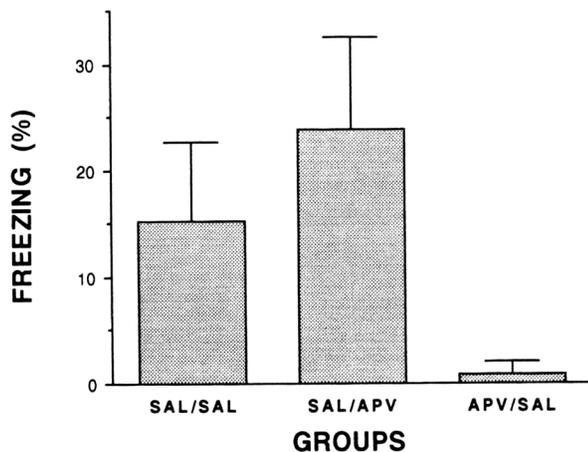


Figure 3. Mean (\pm SE) freezing of SAL ($n = 11$), L-APV ($n = 10$), and racemic APV ($n = 10$) subjects 24 hr after three shocks expressed as the percentage of total observations during the 8-min test.

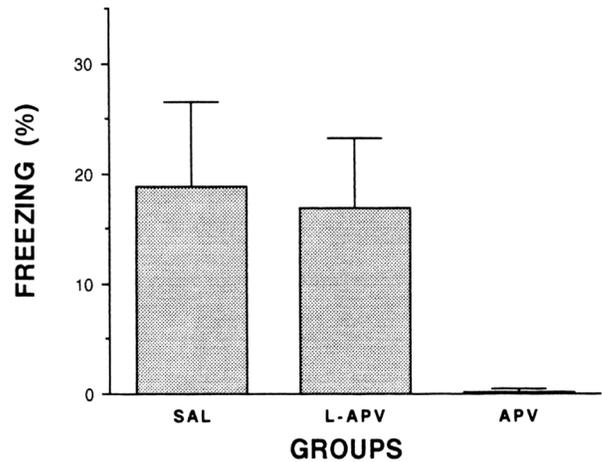


Figure 4. Mean (\pm SE) percentage freezing during the 8-min test of SAL/SAL ($n = 8$), SAL/APV ($n = 8$), and APV/SAL ($n = 8$) groups 24 hr after a single shock.

did not. A one-way ANOVA indicated a significant group difference, $F(2, 23) = 8.18$, $p < .002$.¹ Post hoc analyses (Newman-Keuls) indicated that the APV–SAL group differed reliably from both the SAL–SAL ($p < .05$) and the SAL–APV ($p < .01$) groups. There was no difference between SAL–APV and SAL–SAL groups.

APV blocks the acquisition of fear conditioning only if it is given before the shock. This suggests that APV acts on acquisition or encoding rather than consolidation processes. These findings also indicate that the APV effect obtained in Experiment 1 was not caused by some delayed nonspecific action of the drug.

Experiments 4A and 4B

More painful shocks condition more freezing (e.g., Fanselow & Bolles, 1979). Therefore, if APV reduces pain sensitivity, then it will alter conditioning. There is some plausibility to this interpretation. Fear conditioning activates endogenous analgesic mechanisms that in turn influence fear conditioning (Fanselow, 1986b). NMDA receptors have been shown to play a role in the inhibition of pain (Jacquet, 1988), and infusion of NMDA antagonists into the periaqueductal gray blocks some forms of stress-induced analgesia (Siegfried

¹ Of the 9 animals in group APV–SAL, 8 froze less than 6% of the time. Indeed, 6 of the animals never froze at all. However, 1 rat in this group froze 29% of the time. Although the overall ANOVA including this animal was reliable, the difference between the APV–SAL and the SAL–SAL groups fell just short of statistical significance ($p < .08$). A test for outliers (John & Quenouille, 1977) was performed on the freezing data for all animals, and this animal was found to be a significant outlier. Therefore, the data from this animal were removed from the reported analyses. Data including the outlier were also analyzed with an ANOVA using a square-root transformation of the raw scores and also nonparametrically (Kruskal-Wallis followed by Mann-Whitney U). These alternative analyses lead to identical conclusions.

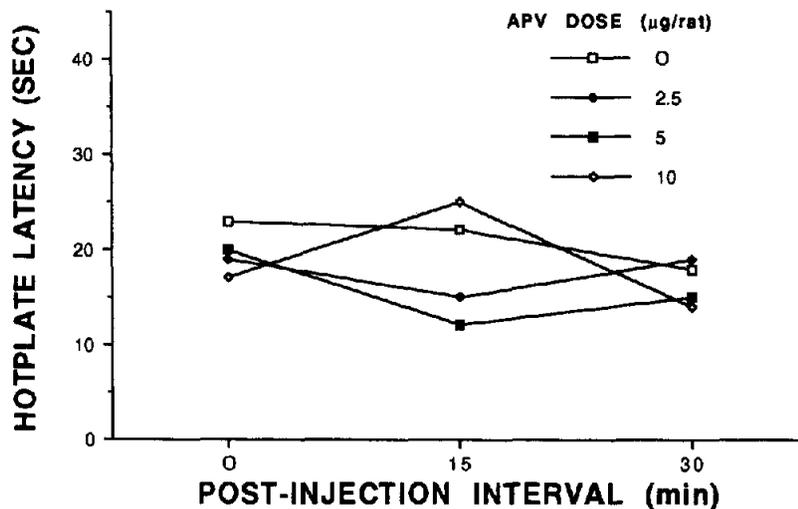


Figure 5. Mean hotplate latency of 0-, 2.5-, 5-, and 10- μ g APV-treated animals at the 0-, 15-, and 30-min postinfusion periods. (Error bars were excluded because they overlapped at all points.)

& Nunes de Souza, 1989). To conclude that APV affected association formation, it is necessary to rule out this possibility. To do so, we used two tests of pain sensitivity. Experiment 4A used the hotplate test, a standard and commonly used test of pain sensitivity. Experiment 4B determined current thresholds for three reactions to shock (flinch, jump, and vocalization). This test was used because, as a nociceptive test stimulus, it used the same stimulus that was used as an unconditional stimulus in the freezing studies.

Method

Subjects. Experiment 4A used 16 naive adult female rats of Long-Evans descent. These animals weighed 222–287 g before surgery and were housed in a manner identical to those animals in Experiment 1. Experiment 4B used the APV and saline subjects of Experiment 2.

Surgery, drug, and injection. These procedures were identical to those of Experiment 1.

Apparatus and procedure. Experiment 4A subjects received icv infusions of either 0 μ g ($n = 4$), 2.5 μ g ($n = 4$), 5 μ g ($n = 4$), or 10 μ g ($n = 4$) of APV. Immediately afterward, rats were placed on a 52 $^{\circ}$ C hotplate (Model 35-D, IITC Inc., Landing, NJ), and paw-lick latency (defined as amount of time it took for a rat to either lick its hind paw or jump vertically with all four paws off the hotplate) was recorded. If the rat did not lick or jump in 45 s, then it was removed and given a latency score of 45 s. All rats were retested on the hotplate at 15 and 30 min postinjection.

Experiment 4B used the apparatus of Experiment 1. The animals were infused with APV (5 μ g/rat) or saline in the same manner as the previous experiments. The animals were assigned to these groups in a manner balanced for their previous experience. Rats were placed in the chamber, and 3 min later they received a train of 0.75-s shocks spaced 10 s apart. The rat was scored as to whether it flinched, jumped, or vocalized to the shock. Flinch was any observable reaction to the shock. This was typically an orienting response of the head usually directed at the grid floor. Jump was any jumping, running, or prancing response to the shock. Vocalization was any audible vocalization to the shock. The shock intensity of the train of shocks

consisted of three ascending series with the following progression: 0.14, 0.28, 0.42, 0.56, 0.70, 0.84, and 0.98 mA. As soon as all three responses were scored in a particular series, the series ended, and the next one began at 0.14 mA. (Shocks greater than 0.98 mA were never needed.) In this manner, three current values were obtained for each response. The experimenter who administered the test was uninformed to the drug conditions.

Histology. An exact procedure to that of Experiment 1 was followed. For Experiment 4A, all subjects showed positive cannula placement. One rat was eliminated from Experiment 4B due to negative cannula placement.

Results and Discussion

Figure 5 represents the hotplate latencies of four groups at different periods postinfusion. As can be seen, animals that received APV infusions at doses of 2.5, 5, or 10 μ g did not differ from the control group in latency to paw lick at any of the time periods measured.

Figure 6 shows the results of the shock threshold test. As expected, flinch had the lowest threshold intensity, with the rats usually showing this reaction to the 2nd or 3rd shock in the ascending series. Mean jump threshold was 66% higher than mean flinch threshold, with vocalization occurring at a slightly higher intensity. Drug effects were analyzed by a separate ANOVA for each measure. As can be seen in Figure 6, there was no drug effect; none of the F values approached significance, F 's (1, 19) = 1.54, 0.35, and 0 for flinch, jump, and vocalization, respectively, all p 's > .2.

These results indicate that APV per se does not alter pain sensitivity. Hence, the APV effect on acquisition of fear conditioning in Experiments 1, 2, and 3 was not due to alteration in pain sensitivity. These data also provide further evidence that APV was not causing either sensory or motor performance deficits; that is, they could lick, jump, flinch, and vocalize normally to sensory stimuli.

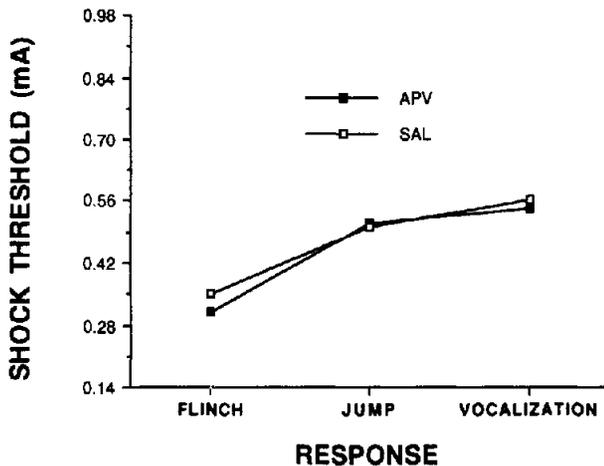


Figure 6. Mean current threshold intensity (mA) to evoke flinch, jump, and vocalization responses in SAL- and APV-pretreated animals. (Error bars were not included because they overlapped at all points.)

General Discussion

The specific NMDA receptor antagonist APV, but not its inactive L enantiomer, prevented the acquisition of conditional fear when administered before training with footshock. Moreover, the behavioral effect of the drug occurred when APV was given before the shock but not immediately after the shock. It is important to note that these effects were detected during tests made in the absence of the drug. Thus, APV affects fear conditioning if it is present at the time when behavioral change (learning) is induced.

To conclude that APV affected learning, it must also be demonstrated that the drug did not impair performance in this task. APV per se did not produce any freezing behavior in untrained animals before shock exposure. Furthermore, APV did not affect the freezing that occurred immediately after shock, even though the same rats showed a freezing deficit the next day. Thus, behavior at the time of training was not compromised. Rats given saline during training but tested in the presence of APV showed normal freezing during the test. In addition, APV did not affect performance on hotplate and shock threshold tasks. Thus, the drug did not significantly compromise sensory or motor functioning. Because APV given immediately after shock had no effect on freezing 24 hr later, the effect of APV cannot be attributed to a delayed nonspecific effect. Clearly APV's effects on conditional responding cannot be attributed to an impairment of performance.

When a drug is given during training, one must be concerned that any decrement in later testing is not simply caused by a change in the stimulus properties of the situation. This would occur if the drug became part of the stimulus context associated with the shock, but the drug state was not recreated during the test. Rats treated with APV during training froze little regardless of whether they were tested with APV or not. Rats trained under saline froze considerably regardless of whether they were tested with or without APV. Therefore,

the reduction in freezing produced by APV cannot be attributed to this sort of state-dependent generalization decrement.

Together these data make a compelling case that APV prevented fear conditioning from occurring. Some additional pilot data provide further evidence toward the specificity of this effect. Using the same 2×2 factorial design, we have found that APV does not affect acquisition, performance, or retention of running for food in a straight alley. The alleyway task that we used produced as rapid learning as our freezing task. Therefore, the effects obtained with APV reported in this article seem to be specific to fear conditioning.

The fact that APV is quite selective for NMDA receptors (Watkins & Olverman, 1987) and that its effect on freezing was stereospecific suggests that NMDA receptors are somehow involved in the learning that occurs during Pavlovian fear conditioning. NMDA receptors are often associated with a form of synaptic plasticity known as LTP (Collingridge, 1985; Collingridge et al., 1983; Muller et al., 1988). As described in the introduction, NMDA and non-NMDA EAA (e.g., quisqualate-kainate) receptors play very different roles in LTP. EAAs acting at the NMDA receptors are responsible for triggering potentiation, but the activity at this receptor itself is not potentiated (Collingridge et al., 1983; Muller et al., 1988). On the other hand, the non-NMDA EAA receptor is potentiated but does not itself induce potentiation (Muller et al., 1988). Thus, the NMDA receptor is important for the induction but not expression of potentiation, whereas the non-NMDA EAA receptor is responsible for the expression but not induction of LTP (Collingridge et al., 1983; Muller et al., 1988). Support for this role of the NMDA receptor is provided by data indicating that APV blocks acquisition but not expression (performance) of LTP (Collingridge et al., 1983; Muller et al., 1988). Therefore, the effects of APV on LTP and fear conditioning are parallel. This suggests that LTP may underly the synaptic changes that occur in fear conditioning.

The present findings can be applied to Pavlovian fear conditioning if one considers a particular form of LTP, associative LTP. In associative LTP, weak synaptic inputs can be potentiated when they are activated at the same time as independent strong synaptic inputs (Brown, Chapman, Kairiss, & Keenan, 1988; Kelso & Brown, 1986). The temporal requirements for the induction of associative LTP appear to be similar to those required to produce associative learning (Levy & Steward, 1983). Pavlovian fear conditioning could occur in the following way: A CS causes release of EAAs onto a postsynaptic cell containing both NMDA and non-NMDA EAA receptors. Activity after the initial CS presentation is weak at non-NMDA receptors because LTP has not yet occurred. In addition, activity at the NMDA receptor-channel complex is normally blocked by extracellular Mg^{2+} ions, thus being impermeable to Ca^{2+} ions, unless the cell is sufficiently depolarized (Mayer, Westbrook, & Guthrie, 1984; Nowak, Bregestovski, Ascher, Herbet, & Prochiantz, 1984). The US need only depolarize the cell at the time the CS was presented to remove the Mg^{2+} blockade of the NMDA receptor-channel complex. Once this happens, the NMDA receptor-channel complex allows Ca^{2+} entry that results in long-lasting changes (LTP) in a later response to EAAs acting on the non-

NMDA receptors (Monaghan et al., 1989). These changes allow the CS to activate the neural systems responsible for freezing. In other words, a neutral stimulus cannot activate the neural structures responsible for producing defensive behavior because the input to those structures is initially too weak. However, with appropriate temporal contiguity, a painful US causes LTP of the input of the CS to these neural structures. If the magnitude of that LTP was proportional to the strength of the US input (Teyler & DiScenna, 1987), then this would also provide an explanation of why stronger USs condition more defensive responses such as freezing (Bolles & Fanselow, 1980; Fanselow, 1986b).

One obvious question is in what neural structures is the LTP that mediates fear conditioning occurring. LTP was first observed and is most associated with the hippocampus but subsequently has been found in many other brain areas as well (Teyler & DiScenna, 1987). However, it is unlikely that the hippocampus is a necessary structure for the simple associative learning reported in this article because simple fear conditioning still occurs after hippocampectomy (Leaton & Borszcz, 1990; Winocur, Rawlins, & Gray, 1987). One area that may be important is the amygdala. The amygdala is a critical structure for fear-related behaviors (e.g., D. C. Blanchard & R. J. Blanchard, 1972; Helmstetter, Leaton, Fanselow, & Calcagnetti, 1988; Hitchcock & Davis, 1986; Kapp, Frysinger, Gallagher, & Haselton, 1979; LeDoux, Iwata, Cicchetti, & Reis, 1988; Pascoe & Kapp, 1985). Recently, LTP has been demonstrated in the amygdala (Clugnet & LeDoux, 1989), and CS pathways that project to the amygdala (LeDoux, Iwata, Pearl, & Reis, 1986) contain EAAs (Farb, LeDoux, & Milner, 1989). Additional support for the hypothesis of amygdala involvement is provided by the recent finding that APV administered directly into the amygdala blocks the potentiation of startle that is caused by fear stimuli (Miserendino, Melia, Sananes, & Davis, 1989). This speculation obviously requires additional testing.

It is interesting to note that freezing observed immediately (20 s) after the shock was unaffected by APV administration. This is somewhat surprising because there are several converging lines of evidence that even the freezing that occurs immediately after shock is entirely a conditional response to contextual cues present at the time of shock administration (Fanselow, 1980, 1982, 1986a). Assuming that freezing immediately after shock is associatively based, then the failure of APV to influence this response suggests the possibility that there is a short-term associative memory not dependent on NMDA receptors. In addition to LTP, brief high-frequency stimulation results in NMDA-independent synaptic potentiation that is of short duration (Collingridge et al., 1983). One can speculate that the very immediate postshock freezing is mediated by this short-lasting synaptic potentiation (e.g., associative posttetanic potentiation) that is not blocked by APV (Collingridge et al., 1983).

In summary, NMDA receptors appear to be critical for the acquisition but play little role in expression of conditional fear. We suggest that endogenously generated NMDA-dependent LTP may be a mechanism underlying the associative changes that occur in fear conditioning. Activity at the NMDA receptor appears essential for the encoding not the consoli-

dation of associative fear memory. In addition, our findings indicate that there may be both long-term and short-term associative memories for fear. We propose that Pavlovian fear conditioning offers a useful behavioral model to explore parallels between learning and LTP.

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