The Effect of the NMDA Antagonist APV on a Spinal Operant Avoidance Task

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ABSTRACT

Previous research has shown that spinal rats can learn a flexion response to avoid shock. Instituting a response-shock contingency could alter behavior by inducing long-term potentiation (LTP), an NMDA-mediated phenomenon. I tested this hypothesis by assessing whether the administration of the NMDA antagonist APV disrupts learning. Subjects were spinally transected at T2 and had catheters inserted to the lumbosacral enlargement. Twenty four hours later, APV (0, 10, 20, or 40 mM) was microinjected, after which subjects received 30 minutes of training. We found that APV disrupted learning of the avoidance response in a dose-dependent fashion.

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Learning is a vital mechanism that allows individuals to adapt to their surroundings and evaluate events based on past experiences. One basic form of learning is known as instrumental, or operant, learning. In this type of learning, an animal acquires information about the relationship between its behavior and its consequences, as when it learns to press a bar to obtain food. Although one might naturally assume that this type of learning depends on neural systems within the brain, recent evidence indicates that lower-level systems within the spinal cord are sensitive to response-reinforcer relations (Grau, Barstow, and Joynes, in preparation).

Instrumental learning within the spinal cord has generally been demonstrated using the Horridge (1962), or master-yoke, paradigm. In this design, a contingency is established between shock and an avoidance response by attaching shock electrodes to the leg of a rat restrained in a tube. Leg position can then be monitored by attaching a rod to the rat's paw and placing a salt solution directly under the rod. The height of the solution is adjusted so that the rod contacts the solution whenever the leg is fully extended. By monitoring this circuit, a response-shock contingency can be established in the master rats by applying shock whenever the rod contacts the solution. To control for the effects

of shock *per se*, other rats are "yoked" to these subjects. Each yoked rat is connected to a master rat so that it receives shock at exactly the same time. For the yoked rats, leg shock is effectively "uncontrollable," for the position of their legs is irrelevant to whether or not shock is applied. Using this procedure, Grau, Barstow, and Joynes (in preparation) and others (Buerger and Fennessy, 1970; Sherman, Hoehler, and Buerger, 1982) have shown that when spinal rats are given control over the occurrence of shock, they rapidly learn to maintain their legs in a flexed position, effectively avoiding the leg shock. In contrast, yoked rats fail to acquire this flexion response. This finding suggests that the spinal cord can support a primitive form of instrumental avoidance learning.

Due to the highly unorthodox nature of the findings, researchers have questioned the validity of the data. Among the more serious challenges was that posed by Church and Lerner (1976) who showed that a simple reactive model could produce differences in the master-yoke paradigm in the absence of learning. According to Church and Lerner (1976), if you want to claim that subjects are sensitive to the response-reinforcer relationship, you need to show that disrupting this relation interferes with learning. To address this criticism, Grau et al. (in preparation) tested whether disrupting response-reinforcer contiguity by interposing a delay in shock onset and offset of 50, 100, or 200 milliseconds disrupted learning. They found that a 50 ms delay attenuated

learning while a 200 ms delay eliminated learning. They have further shown that delaying onset has a much more disruptive effect than delaying offset, which suggests that the behavior is reinforced by shock onset, not offset. In behavioral terms, the response is acquired because it is "punished" by the onset of shock. The reinforcement that could occur upon shock offset, when subjects "escape" from the noxious stimulus, appears to contribute little to the learning.

Relatively little is known about the neurobiological mechanisms that mediate this example of instrumental learning. It does seem to depend on spinal neurons because microinjecting a drug that disrupts neural functioning (lidocaine) prevents learning (Grau et al., in preparation). Interestingly, lidocaine does not eliminate the flexion response. Rather, it eliminates learning by preventing the increase in response duration that normally occurs during training.

The present experiment explores the role of one potential biochemical mechanism that could mediate learning within the spinal cord. This mechanism, which is known as "long term potentiation" (LTP), occurs in some neurons when they are repeatedly stimulated. Neurochemical studies have shown that this phenomenon is mediated by the NMDA receptor (Staubli et al., 1989). This receptor functions as a "gated" channel which is normally blocked by a magnesium ion (Mg^{2+}). Activating the neuron displaces the Mg^{2+} ion and allows excitatory amino acids to

activate the receptor (Dudai, 1989; Foster and Fagg, 1987). This in turn engages a sequence of neuronal events that enhances activity within the neuron so that it is now more likely to fire. Importantly, other studies have shown that the application of a noxious stimulus can induce LTP within the spinal cord (Coderre, Katz, Vaccarino, and Melzack, 1993; Woolf and Thompson, 1991). Given this, we hypothesized that contingent shock might augment the flexion response by inducing LTP. This enhancement of neuronal functioning could then act to maintain the shocked leg in a flexed position.

My experiment tests whether an NMDA-mediated LTP contributes to spinal learning by assessing the impact of the NMDA antagonist 2-amino-5-phosphonopentanoic acid (APV). If LTP contributes, then microinjecting APV into the spinal cord should disrupt the learning in a dose-dependent fashion.

Method

Subjects.

The subjects were 32 male Sprague-Dawley rats obtained from Harlan (Houston, TX). The rats were approximately 90 days old and were individually housed and maintained on a 12 hr light/dark cycle with food and water continuously available.

Apparatus.

Operant training was conducted while spinal rats were loosely restrained in tubes (23.5 cm [length] x 8 cm [internal diameter]). The front of each tube was sealed and the tubes were covered with opaque Duct tape that provided a dark enclosure in which the rats could rest undisturbed. Two slots (5.6 cm [length] x 1.8 cm [width]) were cut 4 cm apart and 1.5 cm from the end of the tube, allowing both hind legs to hang freely. To minimize the effects of upper body movements on leg position, the rat's midsection was gently secured by an insulated wire. This wire was drawn over the rat's dorsolateral surface and passed outside of the tube through two slots spaced 9 cm apart and 8-10 cm from the rear of the tubes.

Leg shock was applied by attaching one lead from a BRS/LVE shock generator (Model SG-903) to a stainless steel wire (0.05 sq. mm [30 AWG]) inserted through the skin over the tibia 1.5 cm from the tarsals. The other lead was attached to a pin that was inserted through the body of the tibialis muscle 1.5 cm above the other

electrode.

Leg position was monitored by attaching a stainless steel rod to the left rear paw. The rod was formed from a 7 cm 0.46 mm stainless steel wire. The last 2.5 cm of the rod was insulated from the paw with heat-shrink tubing. A fine wire (0.01 sq. mm [36 AWG], magnet wire single beldsol) was attached to the end of the rod at a point under the insulation. This wire extended from the rear of the foot and was connected to a digital input board that was monitored by a Macintosh computer. The rod was taped to the plantar surface of the rat's foot with approximately 10 cm of porous tape (Orthaletic, 1.3 cm [width]) with the end positioned directly in front of the plantar protuberance. To minimize lateral leg movements, a piece of porous tape (Orthaletic, 1.3 cm [width]) was wrapped around the leg and taped to a horizontal rod that lay directly under the front panel of the restraining tube. The tape restraint was adjusted so that it was taut enough to extend the joint between the tibia and femur. A plastic rectangular dish (11.5 cm [w] x 19 cm [l] x 5 cm [d]) containing an NaCl solution was placed approximately 7.5 cm below the restraining tube. A drop of soap was added to the solution to reduce surface tension. A ground wire was connected to a stainless steel rod that was placed in the solution. When the rod attached to the rat's paw contacted the solution, a computer-monitored circuit was completed. The status of the circuit was checked approximately 30 times per second by the computer program used to monitor leg position.

Flexion force was measured by looping a monofilament plastic line ("4 lb." test strength, Du Pont) around the rat's ankle. The 40 cm length of line was passed through an eyelet positioned directly under the paw, 16 cm beneath the base of the tube. The end of the line was attached to a strain gauge that was fastened to a ringstand. After the line was secured around the rat's paw, the ringstand was positioned so that the line was taut enough to barely register a force on the gauge. Flexion force was then determined by applying a 3 sec shock to the leg and monitoring the voltage output which was converted to force in Newtons. The shock and force measurement steps were repeated until the desired force deflection of 0.6 N was obtained.

Surgery and histology.

Rats were anesthetized with pentobarbital (40 mg/kg, ip). The surgical procedure required that the rat's head be rendered immobile. This was accomplished by stereotaxic restraint and placement of a small pillow under the subject's chest. After the second thoracic vertebra (T2) was located, an anterior-posterior incision was made and the underlying tissue cleared away from the intervertebral space immediately cranial to T2; spinal transection was then accomplished by cauterization of the visible portion of the cord. Next, a catheter was inserted as described by Yaksh & Rudy (1976). Briefly, a saline-filled sterile polyethylene tube (PE-10) was inserted into the subarachnoid space and gently threaded caudally to the lumbosacral enlargement (9 cm in rats in the age range

employed). Because of the T2 transection, the catheter was externalized and anchored at the thoracic level. After insertion, the intervertebral space was packed with Oxycel (Parke-Davis) and the external wound secured by autoclips. Following closure, each subject's left rear leg was shaven for electrode placement. The rats were then placed in a temperature-controlled environment (25.5 ^{o}C) during the 18-24 hr recovery period.

The transections were confirmed by (i) inspecting the cord during the operation and (ii) observing the behavior of the subjects after they had recovered to ensure that they exhibited paralysis below the level of the forepaws and did not respond to the tailshock used to induce antinociception.

Procedure.

Subjects were randomly assigned to one of four drug conditions (0, 10 mM, 20 mM, or 40 mM APV) before testing. Each subject received the appropriate dose dissolved in 5 ml of saline vehicle (pH 7) followed by 10 ml of saline to flush the catheter 5 minutes prior to testing.

Immediately before testing, each subject's leg was marked for electrode placement. The rats were placed in the restraining tubes and gently secured by the dorsolateral wire belt. After the pin was inserted laterally 0.4 cm into the tibialis muscle at the upper mark, a shock lead was connected to the base of the pin. The other shock lead was connected to the wire that was threaded through the tibialis muscle 1.5 cm below. After the rod used to monitor leg

position was taped to the paw, the lateral movement restraint was taped in place. At this point, shock thresholds were assessed and the shock level was adjusted to produce a 0.6 N deflection for each subject. This shock setting was preserved for the duration of the testing phase. The level of the salt solution was then adjusted by first applying 3 1.5-s legshocks and adjusting the height of the solution so that the tip of the rod lay 2 mm below the surface.

The computer program used to monitor leg position and apply shock for the 30 minutes of testing was then launched. At the end of testing, shock thresholds were measured as described earlier. Upon completion of the experiment, subjects were euthanized with 1.0 ml pentobarbital (40 mg/ml, ip).

Results

The graphs in Figure 1 illustrate the results obtained during the testing period. Subjects administered the saline vehicle exhibited longer response durations (top panel), decreased times in solution (middle panel), and fewer responses (lower panel) as training progressed. The APV antagonist disrupted this learning in a dose dependent fashion.

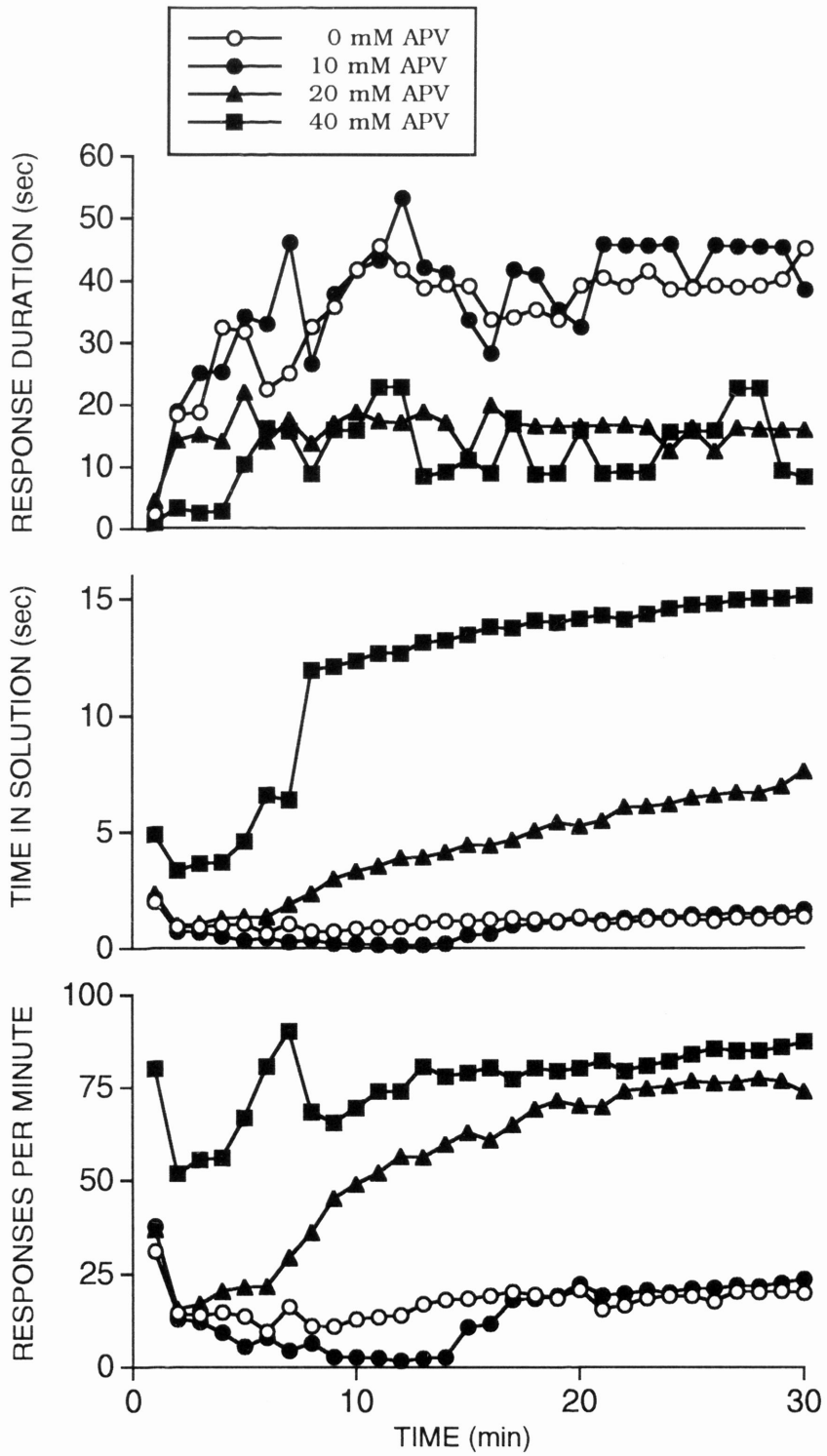

Figure 1. The response duration (top panel), time in solution (middle panel), and response frequency (lower panel) observed in subjects given 0 (open circle), 10 (filled circle), 20 (filled triangle), or 40 mM (filled square) of APV. Each datum point indicates the mean performance observed during a 1 min bin of testing.

The results were analyzed by performing a mixed (4 between [drug dose] by 30 within [sampling bin]) analysis of variance (ANOVA) on each dependent variable. The analysis of the response duration data indicated that the drug had a significant impact on performance, $F(3,28) = 3.31$, $p < .05$. Although a significant trials effect existed $F(29, 812) = 4.65$, $p < .001$, the trial by drug interaction was not significant, $F(87,812) = .88$, $p > .05$. Trend analysis was then used to further characterize the between-subjects differences. These analyses revealed a significant linear component, $F(1,28) = 7.94$, $p < .01$. Neither the quadratic nor cubic components were significant, as both Fs < 1.78, $p > .05$.

A similar analysis of the times in solution failed to find an overall effect of drug treatment, $F(3,28) = 2.80$, $p > .05$. However, trend analysis revealed a significant linear component, $F(1,28) = 6.78$, $p < .05$. Neither the quadratic nor the cubic components were significant, Fs < 1.62, $p > .05$. Both the trials effects and the trials by drug interaction were significant, both Fs > 1.82, $p < .001$.

An ANOVA performed on the number of responses failed to find a significant effect of drug treatment, $F(3,28) = 2.82$, $p > .05$, but again trend analysis revealed a significant linear trend, $F(1,28) = 7.39$, $p < .05$. Neither the quadratic nor cubic trends proved to be significant, both Fs < 1.0, $p > .05$. Although there was a significant trials effect, $F(29,812) = 3.82$, $p < .001$, the trial by drug interaction was not significant, $F(87,812) = 1.11$, $p > .05$.

Figure 2 depicts the shock thresholds as a function of drug

dose. It is clear that the groups did not differ prior to training, $F(3,28) = 1.39$, $p > .05$. After training, rats that failed to learn, and consequently received more shock, exhibited longer latencies $F(3,28) = 3.16$, $p < .05$. Post hoc comparisons with Duncan's multiple range test revealed that the subjects given 40 mM of APV exhibited higher thresholds relative to the groups that received either 0 or 10 mM of APV. No other differences were significant.

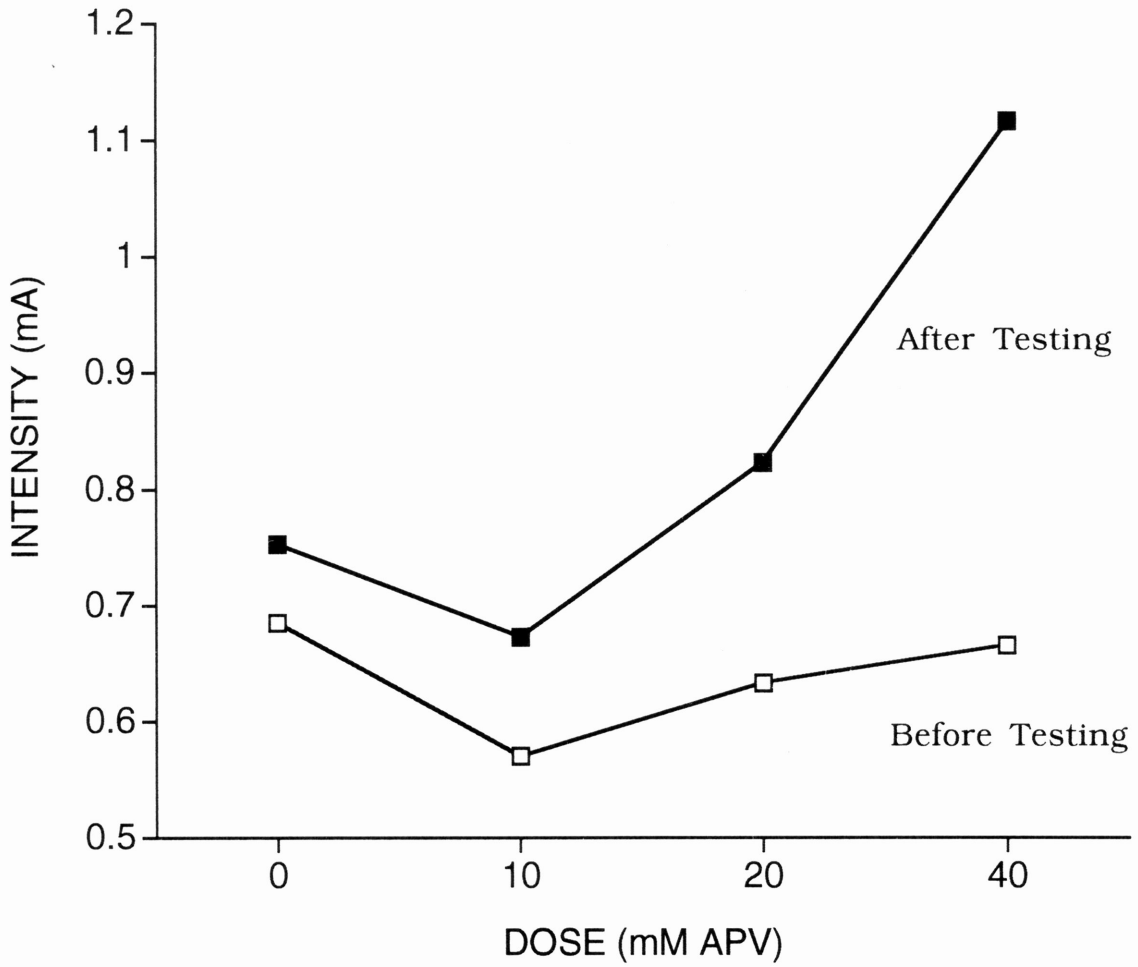

Figure 2. The shock intensity needed to elicit a flexion response force of 0.6 N before (open squares) and after (filled squares) testing in subjects given 0, 10, 20, or 40 mM of APV.

General Discussion

My experiment was designed to explore the role of the NMDA receptor and LTP in instrumental learning at the level of the spinal cord. I addressed this issue by testing the impact of the NMDA antagonist APV. As expected, the vehicle controls acquired the instrumental response. This learning was evident from the increase in response duration that occurred as a function of training. As response duration increased, subjects spent less time in the solution and exhibited fewer responses. The NMDA antagonist APV disrupted this learning in a dose-dependent fashion, and this effect was evident on each of the behavioral measures. These results suggest that the NMDA receptor and LTP play a role in instrumental learning at the level of the spinal cord.

What my findings imply is that contingent shock strengthens the flexion response by inducing LTP within the spinal cord. By enhancing the efferent output to the muscles that produce the flexion response, this LTP could produce a systematic increase in response duration. However, a shock-induced LTP cannot, by itself, explain all of the results. One difficulty is that yoked rats fail to acquire the flexion response even though they receive the same shocks as the master rats. Moreover, when rats that have experienced inescapable shock are given contingent shock, they fail to learn (Chopin and Bennett, 1974; Grau et al., in preparation), a phenomenon that is reminiscent of the "learned helplessness"

effect frequently found in intact subjects (Maier and Seligman, 1976). This suggests that exposure to noncontingent shock not only fails to induce LTP, it prevents its later induction when a response-shock contingency is instituted.

These observations force one to conclude that additional changes, beyond the simple induction of LTP by shock (Woolf and Thompson, 1991), contribute to the master-yoke difference; whereas contingent shock enhances the flexion response, noncontingent shock weakens the response and hurts learning when a response-shock contingency is later instituted. Accounting for these results will require two processes, one of which is likely to be an NMDA-mediated sensitization of the flexion response. The other process must somehow undermine learning and performance, either through the depletion of a critical neurotransmitter (e.g., an excitatory amino acid) or by the induction of an inhibitory process (e.g., an opioid antinociception). One could evaluate these alternative accounts by testing whether the helplessness-like effect observed after noncontingent shock is eliminated by either replacing the depleted excitatory amino acid or blocking opioid function with selective antagonists.

Notice, though, that if shock alone engaged these facilitory and inhibitory mechanisms, a master-yoke difference would not emerge. To obtain such a difference, one of the mechanisms must depend on the response-reinforcer relation. In behavioral terms, the magnitude of at least one process must depend on leg position.

One potential explanation for how such a response dependency could be established can be derived from casual observation of the rats' behavior during testing. Master rats receive shock while their muscles are still partially flexed, presumably while the leg muscles are still receiving some suprathreshold efferent impulses. In contrast, yoked rats, which characteristically exhibit learned helplessness, often receive shock after their legs have already attained full flexion and are relaxed. Because LTP is greater in neurons that are already active (Dudai, 1989), the escape response elicited by master rats may be preferentially enhanced, strengthening the flexion response. The comparative lack of efferent activity in yoked rats at the time shock is received may preclude the generation of an "LTP enhancement effect."

My findings extend the generality of the role of NMDA receptor and LTP in neural functioning. Because LTP was first described in hippocampal neurons, researchers thought it mediated higher cognitive functioning and spatial memory. The present findings, along with other work (Woolf and Thompson, 1991) indicate that the NMDA receptors and LTP may play a more widespread role in learning and memory than was once thought, a role that extends across all levels of the nervous system (Raigorodsky and Urca, 1987; Morris, 1990). In fact, recent evidence indicates that an NMDA-mediated LTP even contributes to learning in some simple invertebrates (e.g., *Aplysia*) which implies that this mechanism evolved very early and has been highly

conserved (Walters, 1994).

In summary, my results suggest that LTP and the NMDA receptor play a critical role in the development of an instrumental response in spinal rats. Further research is needed to evaluate other components of LTP (e.g., NO) and to identify the inhibitory mechanisms that produce the helplessness-like effect in rats given noncontingent shock.

References

Bliss, T.V.P., and Gardner-Medwin, A.R. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the unanesthetized rabbit following stimulation of the perforant path. *Journal of Physiology, 232*, 357-374.

Bliss, T.V.P., and Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anesthetized rabbit following stimulation of the perforant path. *Journal of Physiology, 232*, 331-356.

Buerger, A. A., and Fennessy, A. (1970). Long-term alteration of leg position due to shock avoidance by spinal rats. *Nature, 225*, 751-752.

Chopin, S.F., and Bennett, M.H. (1974). The effect of unavoidable shock on instrumental avoidance conditioning in spinal rats. *Physiology and Behavior, 14*, 399-401.

Church, R.M., and Lerner, N.D. (1976). Does the headless cockroach learn to avoid? *Physiological Psychology, 4*, 439-442.

Coderre, T.J., Katz, J., Vaccarino, A.L., Melzack, R. (1993). Contribution of central neuroplasticity to pathological pain: review of clinical and experimental evidence. *Pain, 52*, 259-285.

Dickenson, A.H. (1990). A cure for wind up: NMDA receptor antagonists as potential analgesics. *Trends in Pharmacological Science, 11*, 307-309.

Foster, A.C., and Fagg, G.E. (1987). Taking apart NMDA receptors. *Nature, 329*, 395-396.

Horridge, G.A. (1962). Learning of the leg position by the ventral nerve cord in headless insects. *Proceedings of the Royal Society, 157*, 33-52.

Keith, J.R., and Rudy, J.W. (1990). Why NMDA-receptor dependent long-term potentiation may not be a mechanism of learning and memory: Reappraisal of the NMDA-receptor blockade strategy. *Psychobiology, 18*, 251-257.

Maier, S.F. and Seligman, M.E.P. (1976). Learned helplessness: Theory and evidence. *Experimental Psychology, 105*, 3-46.

Morris, R.G.M., Anderson, E., Lynch, G.S., and Baudry, M. (1986). Selective impairment of learning and blockade of long-term potentiation by an *N*-methyl-D-aspartate receptor antagonist, AP5. *Nature, 319*, 774-776.

Morris, R.G.M. (1990). It's heads they win, tails I lose! *Psychobiology, 18*, 261-266.

Raigorodsky, G., and Urca, G. (1987). Intrathecal *N*-methyl-D-aspartate (NMDA) activates both nociceptive and antinociceptive systems. *Brain Research, 422*, 158-162.

Robinson, G.S., Jr., Crooks, G.B., Jr., Shinkman, P.G., and Gallagher, M. (1989). Behavioral effects mimic deficits associated with hippocampal damage. *Psychobiology, 17*, 156-164.

Sherman, B.S., Hoehler, F.K., Buerger, A.A. (1982). Instrumental avoidance conditioning of increased leg lowering in the spinal rat. *Physiology and Behavior, 25*, 123-128.

Staubli, U., Thibault, O., DiLorenzo, M., and Lynch, G. (1989). Antagonism of NMDA receptors impairs acquisition but not retention of olfactory memory. *Behavioral Neuroscience, 103*, 54-60.

Walters, E.T. (1994). Injury-related behavior and neuronal plasticity: an evolutionary perspective on sensitization, hyperalgesia, and analgesia. *International Review of Neurobiology, 36*, 325-427.

Woolf, C.J., and Thompson, S.W.N. (1991). The induction and maintenance of central sensitization is dependent on *N*-methyl-D-aspartic acid receptor activation; implications for the treatment of post-injury pain hypersensitivity states. *Pain, 44*, 293-299.

Yaksh, T.H., and Rudy, T.A. (1976). Chronic catheterization of the spinal cord subarachnoid space. *Physiology and Behavior, 17*, 1031-1036.