Opioid Receptors Regulate the Extinction of Pavlovian Fear Conditioning

Gavan P. McNally and R. Frederick Westbrook
University of New South Wales

Rats received a single pairing of an auditory conditioned stimulus (CS) with a footshock unconditioned stimulus (US). The fear (freezing) that had accrued to the CS was then extinguished. Injection of naloxone prior to this extinction significantly impaired the development of extinction. This impairment was mediated by opioid receptors in the brain and was not observed when naloxone was injected after extinction training. Finally, an injection of naloxone on test failed to reinstate extinguished responding that had already accrued to the CS. These experiments show that opioid receptors regulate the development, but not the expression, of fear extinction and are discussed with reference to the roles of opioid receptors in US processing, memory, and appetitive motivation.

Activity at opioid receptors modulates the acquisition of Pavlovian aversive conditioning. Systemic or intracerebral administrations of opioid receptor agonists typically impair, whereas such administrations of opioid receptor antagonists facilitate, acquisition of aversive conditioning across a variety of species (e.g., rat, rabbit, mouse) and conditioning preparations (e.g., freezing, heart rate, eyeblink; for a review, see Fanselow, 1998). Opioid peptides in the mammalian central nervous system (CNS) modulate a number of sensory, motivational, and cognitive functions (for a review, see McNally & Akil, 2002). It is perhaps not surprising, therefore, that there are multiple mechanisms mediating opioid modulation of aversive conditioning.

Consistent with a role of opioid receptors in antinociception, there is evidence that the release of endogenous opioid peptides following presentation of either an aversive unconditioned stimulus (US) or a conditioned stimulus (CS) previously paired with such a US constitutes an important mechanism for modulating the motivational (Schull, 1979) and sensory (Bolles & Fanselow, 1980) impact of the US. This evidence includes demonstrations that increases in shock intensity and naloxone pretreatment have similar effects on conditioning (Young & Fanselow, 1992), post-shock activity (Fanselow, 1984), and shock-elicited defensive fighting (Fanselow & Sigmundi, 1982; Fanselow, Sigmundi, & Bolles, 1980), as well as demonstrations that naloxone facilitates conditioning based on multiple CS–US pairings but not a single CS–US pairing (Young & Fanselow, 1992). Opioid modulation of aversive conditioning can be understood in terms of activation of antinociceptive mechanisms that modulate perceived intensity of the US. Specifically, opioid agonists disrupt US processing by inhibiting the detection of the painful US at the level of the spinal

cord (antinociception), whereas opioid antagonists facilitate this processing (pronociception). In effect, opioid agonists reduce, whereas antagonists increase, the functional intensity of an aversive US such as shock.

Consistent with a role of opioid receptors in memory processing, posttraining administrations of opioid receptor agonists impair (e.g., Introini, McGaugh, & Baratti, 1985), whereas posttraining administrations of opioid receptor antagonists facilitate (e.g., Introini-Collison, Nagahara, & McGaugh, 1989; McGaugh, Introini-Collison, & Nagahara, 1988), retention of aversive conditioning indexed by passive avoidance. However, unlike the effects of pretraining injections of naloxone on the acquisition of conditioned freezing described above, these posttraining effects of naloxone on retention of passive avoidance frequently do not interact with US intensity. Moreover, these facilitatory effects of naloxone have been observed in spatial learning tasks such as the radial arm maze (e.g., Bostock, Gallagher, & King, 1988; Gallagher, King, & Young, 1983) and in learning about the absence of an aversive US, such as in latent inhibition of eyeblink conditioning in rabbits (e.g., Gallagher, Meagher, & Bostock, 1987). Together, these findings show that the release of opioid peptides during and immediately following a conditioning episode impairs memorial processing of that episode. Opioid antagonists either prevent this reactive opioid inhibition of memory or increase memory consolidation, and thereby facilitate conditioning (for a review, see Gallagher, 1989). Further evidence consistent with a memory-based role for opioid function is provided by demonstrations that administration of opioid receptor antagonists prevents the amnesic effects of social isolation (Rudy, Kuwagama, & Pugh, 1999), hippocampal stimulation (Collier, Quirk, & Routtenberg, 1987), amygdala stimulation (Liang, Messing, & McGaugh, 1983), electroconvulsive shock (Carrasco, Dias, & Izquierdo, 1982), protein synthesis inhibitors (Kameyama, Nabeshima, & Kozawa, 1986), phencyclidine (Nabeshima, Kozawa, Furukawa, & Kameyama, 1986), and scopolamine (Rush, 1986).

Considerable evidence suggests that extinction promotes new learning, rather than the loss of the old, and that this new learning serves to interfere with expression of the old learning (for reviews, see Rescorla, 2001). Hence, opioid receptor manipulations could also be expected to modulate the acquisition and/or consolidation of extinction memories. However, there have been few investiga-

Gavan P. McNally and R. Frederick Westbrook, School of Psychology, University of New South Wales, Sydney, New South Wales, Australia.

The experiments and preparation of the article were supported by Grants DP0343808 and A00103609 from the Australian Research Council and by Grant 209577 from the National Health and Medical Research Council. We thank Marika Gorissen for assistance with these experiments.

Correspondence concerning this article should be addressed to Gavan P. McNally, School of Psychology, University of New South Wales, Sydney, New South Wales 2052, Australia. E-mail: g.mcnelly@unsw.edu.au

tions of this possibility, and the available evidence is conflicting. For example, Hernandez and Powell (1980) reported that intravenous (iv) administrations of naloxone prior to 30 nonreinforced presentations of the CS impaired development of extinction in a rabbit eyeblink conditioning preparation. Similarly, Hernandez and Powell (1983) provided evidence that iv administrations of naloxone impaired extinction of aversive conditioning in the rabbit, as measured by heart rate. This ability of naloxone to impair extinction of aversive conditioning is interesting because it contrasts with the drug's ability to enhance the acquisition of such conditioning. However, Vigorito and Ayres (1987) failed to detect any influence of a systemic injection of naloxone during extinction of fear in rats, as indexed by conditioned suppression. Therefore, the role of opioid receptors in the extinction of aversive conditioning remains unclear.

The experiments reported here studied the role of opioid receptors in the extinction of Pavlovian aversive conditioning in rats, as indexed by the species-specific defense response of freezing. In each of the experiments, rats were first subjected to a single pairing of an auditory CS with a footshock US. The fear reactions (freezing) elicited by the CS were then extinguished by nonreinforced presentations of the CS. Finally, the fear reactions elicited by the CS were tested. Experiment 1 examined the effects of a systemic injection of naloxone on the extinction of Pavlovian fear conditioning across prolonged extinction training. Experiment 2 examined the role of peripheral versus central opioid receptors in the extinction of Pavlovian fear conditioning. Experiment 3 examined the effects of postextinction injections of naloxone. Finally, Experiment 4 examined whether an injection of naloxone on test would reinstate extinguished fear.

Experiment 1

The aim of Experiment 1 was to characterize the effects of opioid receptor antagonism on the extinction of Pavlovian fear conditioning in rats, as indexed by the species-specific defense response of freezing. The design was a 2×2 factorial in which the first factor refers to the type of injection given prior to extinction sessions (saline vs. naloxone) and the second factor refers to the presence versus the absence of the footshock US during conditioning (shocked vs. not shocked). The two unshocked control groups (Groups No Shock-Saline and No Shock-Naloxone) were included to determine whether injections of naloxone in the absence of the footshock US would elicit freezing. On Day 1, rats received a single presentation of the auditory CS, which coterminated with shock according to group allocations. Across the following 4 days, the freezing reactions to the auditory CS were extinguished by means of six daily nonreinforced exposures. These extinction sessions were preceded by injections of naloxone or saline, according to group allocations.

Method

Subjects. The subjects were 32 experimentally naive, adult male Wistar rats (280–350 g) obtained from a commercial supplier (Gore Hill Research Laboratories, Sydney, Australia). After arrival, rats were housed in groups of 6–8 in plastic cages maintained on a 12-hr light–dark cycle (lights on at 7 a.m.) and were allowed access to water and food ad libitum. The rats were handled (10–20 s per rat per day) for 3 days to habituate them to the experimenter. There were 8 rats per group. The procedures

used in these experiments were conducted in accordance with the Guidelines for Ethical Conduct in the Care and Use of Animals (American Psychological Association) and were approved by the Animal Care and Ethics Committee at The University of New South Wales.

Apparatus. Conditioning, extinction, and testing were conducted in a set of four identical chambers (24 cm long $\times$ 30 cm wide $\times$ 24 cm high). The front and rear walls and hinged lids of these chambers were constructed of clear Perspex, and the end walls were made of stainless steel. The floor in each chamber consisted of stainless steel rods, 4 mm in diameter, spaced 15 mm apart (center to center). Each chamber stood 2 cm above a tray of paper pellet bedding (Fibrecycle, Mudgeeraba, Australia) that was changed between rats. These four chambers were located individually within sound-attenuating boxes. The US was a 1-s, 0.8-mA unscrambled AC 50-Hz shock from a constant-current generator, delivered to the floor of each chamber. The current available to each floor could be adjusted with an in-line milliampere meter. The CS was a 74-dB (A scale) 20-Hz clicker delivered through speakers mounted in the lid of each box. Digital video cameras were mounted on the rear wall of each box and connected to a digital multiplexer in an adjacent room, which in turn was connected to a video recorder. The stimuli used for conditioning were controlled by computer (LabView; National Instruments, Austin, TX).

Drugs. Naloxone hydrochloride (Sigma Chemical, St. Louis, MO) was dissolved in 0.9% (wt/vol) nonpyrogenic saline to obtain a concentration of 2.5 mg/ml. This 0.9% nonpyrogenic saline was used for control injections. All injections were given subcutaneously (sc) in the dorsal neck region, in a volume of 1 ml/kg.

Procedure. On Day 1, rats were transported in squads of 4 and placed in the conditioning chambers. Two minutes later, the 10-s CS was presented and coterminated with delivery of shock for rats in Group Shock-Saline and Group Shock-Naloxone. Rats remained in the chambers for a further 50 s before being returned to their home cage. On Day 2, rats were transported to the laboratory and injected with naloxone or saline according to their group allocations. Twenty minutes later, they were placed in the conditioning chamber. After 2 min, the auditory CS was presented for 2 min. CS presentation was repeated a further five times at 2-min interstimulus intervals, so that by the end of this session rats had received six nonreinforced 2-min presentations of the CS, each separated by 2 min. This injection and extinction procedure was repeated on Days 3, 4, and 5 for rats in Groups Shock-Saline and Shock-Naloxone. Thus, at the end of extinction, rats in Groups Shock-Saline and Shock-Naloxone had received a single CS-US pairing and 24 nonreinforced presentations of the CS, under the influence of either saline or naloxone.

Statistics. In this and remaining experiments, performance during extinction and test was videotaped. The rats were subsequently scored as either freezing (defined as the absence of all movement other than that required for breathing) or not freezing every 4 s. The levels of pre-CS freezing were calculated for the minute immediately preceding the first CS presentation. The percentage of these observations scored as freezing was then calculated. The videotapes were scored by two observers, one of whom was blind to a rat's group allocation. The interrater correlations consistently exceeded .85. The data were analyzed by means of a planned orthogonal contrast testing procedure, adopting a multivariate approach to repeated measures where necessary (O'Brien & Kaiser, 1985). The Type I error rate was controlled at .05 for each contrast tested.

Results

The mean ($\pm$ SEM) percentage of observations scored as freezing across the 4 days of extinction are shown in Figure 1. Inspection of these panels suggests that sc injection of naloxone prior to extinction training impaired the development of extinction, as indexed by higher levels of freezing across each of the extinction sessions, compared with injection of saline. This impairment did not appear to be due to the elicitation of freezing by sc naloxone,

Figure 1. Mean ($\pm$ SEM) percentage of observations scored as freezing across four sessions of extinction in Experiment 1. Rats received a subcutaneous injection of either 2.5 mg/kg naloxone or saline prior to each extinction session. For some data, SEMs are too small for error bars to be visible.

because rats in Groups No Shock–Saline and No Shock–Naloxone both showed low levels of freezing during the first extinction session. These observations were confirmed by the statistical analysis.

During the first extinction session, there was a significant overall difference in levels of pre-CS freezing between rats in Groups No Shock–Saline and No Shock–Naloxone versus Groups Shock–Saline and Shock–Naloxone, $F(1, 28) = 66.4, p < .01$. However, there was no significant difference in levels of pre-CS freezing between Group No Shock–Saline versus Group No Shock–Naloxone, $F(1, 28) < 1, p > .05$, nor was there a significant difference in levels of pre-CS freezing between Group Shock–Saline versus Group Shock–Naloxone, $F(1, 28) = 1.0, p > .05$. Collapsed across rats in Groups Shock, a within-subjects t test revealed a significant increase in freezing from the pre-CS period to the first CS presentation ($p = .02$). Overall, rats in Groups Shock–Saline and Shock–Naloxone displayed significantly more freezing across the six CS presentations compared with rats in Groups No Shock–Saline and No Shock–Naloxone, $F(1, 28) = 259.6, p < .01$. Rats in Group Shock–Naloxone displayed significantly more freezing than rats in Group Shock–Saline, $F(1, 28) = 12.9, p < .01$, whereas rats in Group No Shock–Naloxone did not differ significantly from rats in Group No Shock–Saline in levels of freezing, $F(1, 28) < 1, p > .05$. There was an overall linear decrease in freezing across the six nonreinforced presentations of the CS, $F(1, 28) = 12.6, p < .01$. This linear decrease in freezing did not interact with the differences between the rats in Groups No Shock versus Groups Shock, $F(1, 28) = 2.4, p > .05$, nor did it interact with the difference between rats in Group No Shock–Saline versus Group No Shock–Naloxone, $F(1, 28) < 1, p > .05$. However, this linear decrease did interact with the difference between rats in Group Shock–Saline versus Group Shock–Naloxone, $F(1, 28) = 15.5, p < .01$, such that the decrease in freezing across CS presentations was significantly greater among rats in Group Shock–Saline as compared with rats in Group

Shock–Naloxone. Thus, sc injection of naloxone impaired the extinction that otherwise would have accrued to the auditory CS.

During the second extinction session, there was again no significant difference in levels of pre-CS freezing between rats in Group Shock–Saline versus rats in Group Shock–Naloxone, $F(1, 14) < 1, p > .05$. Collapsed across rats in Groups Shock, a within-subjects t test revealed a significant increase in freezing from the pre-CS period to the first CS presentation ($p < .01$). There was, however, an overall significant difference in levels of freezing between these groups across the six nonreinforced CS presentations, with significantly greater levels of freezing among rats in Group Shock–Naloxone, $F(1, 14) = 42.6, p < .01$. There was an overall significant linear decrease in freezing across the extinction session, $F(1, 14) = 20.3, p < .01$. However, this decrease did not interact with the difference between Group Shock–Saline and Group Shock–Naloxone, $F(1, 14) < 1, p > .05$.

During the third extinction session, there was a significant difference in levels of pre-CS freezing between rats in Group Shock–Saline and rats in Group Shock–Naloxone, $F(1, 14) < 5.8, p = .03$, such that there was significantly greater freezing among rats in Group Shock–Naloxone. Collapsed across rats in Groups Shock, a within-subjects t test failed to reveal a significant increase in freezing from the pre-CS period to the first CS presentation ($p > .05$). There was an overall significant difference in levels of freezing between these groups across the six nonreinforced CS presentations such that, again, there were significantly greater levels of freezing among rats in Group Shock–Naloxone, $F(1, 14) = 26.8, p < .01$. There was no overall significant linear decrease in freezing across the extinction session, $F(1, 14) = 1.4, p > .05$. There was also no interaction between this linear decrease and the difference between Group Shock–Saline and Group Shock–Naloxone, $F(1, 14) < 1, p > .05$.

During the fourth and final extinction session, there was again a significant difference in levels of pre-CS freezing between rats in Group Shock–Saline and rats in Group Shock–Naloxone, $F(1,$

14) = 15.4, $p < .01$, such that there was significantly greater freezing among rats in Group Shock–Naloxone. Collapsed across rats in Groups Shock, a within-subjects t test failed to reveal a significant increase in freezing from the pre-CS period to the first CS presentation ($p > .05$). There was also an overall significant difference in levels of freezing between these groups across the six nonreinforced CS presentations such that there was significantly more freezing among rats in Group Shock–Naloxone, $F(1, 14) = 21.2$, $p < .01$. There was no significant linear decrease in freezing across the extinction session, $F(1, 14) = 3.5$, $p > .05$. There was also no interaction with the difference between Group Shock–Saline and Group Shock–Naloxone, $F(1, 14) = 1.0$, $p > .05$.

Discussion

This experiment has revealed that sc injection of naloxone prior to nonreinforced presentations of an auditory CS previously paired with shock significantly impairs the extinction that otherwise accrues to that CS. Thus, freezing to the CS was abolished by the end of the second extinction session among saline-treated rats (i.e., after 12 nonreinforced exposures). In contrast, this freezing was still apparent among naloxone-treated rats during the fourth extinction session and reached low levels only at the end of that extinction session (i.e., after 24 nonreinforced exposures). This impairment of extinction by naloxone was also observed in terms of levels of contextual, or pre-CS, freezing during the third and fourth extinction sessions. These impairments in contextual and CS fear extinction cannot be attributed to any tendency of naloxone to elicit freezing per se, because rats injected with naloxone but not subjected to the footshock US did not differ in levels of freezing from similarly nonshocked rats injected with saline. However, interpretation of levels of CS freezing during Days 3 and 4 of extinction is complicated by the absence of a significant increase in freezing from the pre-CS period to the first CS presentation. Regardless, the important finding from this experiment is that actions at opioid receptors are critical for the extinction of Pavlovian fear conditioning.

Experiment 2

Experiment 2 had two aims. The first aim was to replicate the finding of Experiment 1: that antagonism of opioid receptors impairs the development of fear extinction in rats, as indexed by the species-specific defense response of freezing. The second aim was to identify the locus of the opioid receptors critical for modulation of extinction. The evidence reviewed in the introduction is consistent with the possibility that opioid receptors in the CNS modulate Pavlovian fear conditioning. However, there is evidence that the actions of opioid peptides at their receptors in the peripheral nervous systems also regulate Pavlovian conditioning. For example, Rudy et al. (1999) have shown that actions at peripheral opioid receptors can impair the acquisition of Pavlovian fear conditioning in rats. Similarly, Hernandez, Watson, Fowler, Bair, and Singha (1997) have shown that antagonist actions at peripheral opioid receptors can facilitate the acquisition of aversive conditioning in the rabbit, as indexed by conditioned alterations in heart rate. These findings raise the possibility that the effects of naloxone on the extinction of conditioned fear might be mediated by the actions of the drug in the periphery rather than in the CNS.

Experiment 2 studied the effects of peripheral (naloxone methiodide) versus central and peripheral (naloxone hydrochloride) opioid receptor antagonism on extinction of Pavlovian fear conditioning. The experiment used a single-factor design such that rats were allocated to one of the following three groups: Group Saline, Group Naloxone Methiodide, and Group Naloxone.

Method

Subjects. The subjects were 21 experimentally naive, adult male Wistar rats (280–350 g) obtained from the same source and maintained under the same conditions as described previously. There were 7 rats per group.

Apparatus. Conditioning, extinction, and testing were conducted in a set of four identical chambers (20 cm long $\times$ 21 cm wide $\times$ 23 cm high) that were different from those described in Experiment 1. The front and rear walls and lids of these chambers were made of clear Perspex, and the end walls were made of stainless steel. The floor in each chamber consisted of stainless steel rods, 2 mm in diameter, spaced 10 mm apart (center to center). The US was a 1-s, 0.8-mA unscrambled AC 50-Hz shock from a constant-current generator, delivered to the floor of each chamber. The current available to each floor could be adjusted by means of an in-line milliamperemeter. The CS was a 74-dB (A scale) 20-Hz clicker delivered through a speaker mounted in the ceiling of the experimental room. Each chamber stood 5 cm above a tray of paper pellet bedding (FibreCycle) that was changed between rats. These four chambers were located within separate compartments of a wooden cabinet. A video camera was mounted on the wall of the experimental room and connected to a video recorder in an adjacent laboratory. The stimuli used for conditioning were controlled by computer (LabView).

Drugs. Naloxone hydrochloride and naloxone methiodide (both from Sigma Chemical) were dissolved in 0.9% (wt/vol) nonpyrogenic saline to obtain concentrations of 2.5 and 5.0 mg/ml, respectively. This 0.9% nonpyrogenic saline was used for control injections. All injections were given sc in the dorsal neck region, in a volume of 1 ml/kg.

Procedure. On Day 1, rats were transported in squads of 4 and placed in the conditioning chambers. Two minutes later, the 10-s CS was presented and coterminated with delivery of shock. Rats remained in the chambers for a further 50 s before being returned to their home cage. On Day 2, rats were transported to the laboratory and injected with saline, naloxone, or naloxone methiodide according to their group allocations. They were placed in the conditioning chambers 20 min later. The procedure for extinction was the same as that described in Experiment 1. Six hours later, in the home cages, rats that had been injected with saline or naloxone methiodide were injected with naloxone, whereas rats that had been injected with naloxone were injected with saline. The purpose of these home cage injections was to equate the groups for exposure to an opioid receptor antagonist. On Day 3, rats were transported to the laboratory and placed in the conditioning chambers. The 2-min auditory CS was presented 2–4 and 6–8 min after placement in the chamber.

Results

The mean ($\pm$ SEM) percentage of observations scored as freezing during extinction (Day 2) and test (Day 3) are shown in the left and right panels, respectively, of Figure 2. Inspection of these panels suggests that sc injection of 2.5 mg/kg naloxone prior to extinction training impaired the development of extinction, as indexed by higher levels of freezing during extinction and on test, as compared with saline injection. By contrast, the sc injection of 5.0 mg/kg naloxone methiodide failed to affect levels of freezing. These observations were confirmed by the statistical analysis.

Figure 2. Mean ($\pm$ SEM) percentage of observations scored as freezing across extinction (left panel) and test (right panel) in Experiment 2. Rats received an injection of either 2.5 mg/kg naloxone, 5.0 mg/kg naloxone methiodide, or saline prior to extinction training. For some data, SEMs are too small for error bars to be visible.

There were no differences between groups in levels of pre-CS freezing during extinction, $F(1, 18) < 1, p > .05$. Collapsed across groups, a within-subjects t test confirmed that there was a significant increase in freezing on the first CS presentation as compared with the pre-CS period ($p = .02$). During extinction training, there was a significant overall difference in levels of freezing between rats in Group Naloxone versus rats in Groups Saline and Naloxone Methiodide, $F(1, 18) = 18.8, p < .01$. There was no significant overall difference in levels of freezing between rats in Group Naloxone Methiodide and rats in Group Saline, $F(1, 18) < 1, p > .05$. There was an overall linear decrease in freezing across the six nonreinforced presentations of the CS, averaged across groups, $F(1, 18) = 75.4, p < .01$. Moreover, this linear decrease in freezing interacted with the differences between rats in Group Naloxone versus rats in Groups Naloxone Methiodide and Saline, $F(1, 18) = 20.7, p < .01$. This interaction shows that the decrease in freezing across the extinction session was significantly greater among rats in Groups Saline or Naloxone Methiodide as compared with rats in Group Naloxone. However, this linear decrease did not interact with the differences between rats injected with saline and rats injected with naloxone methiodide, $F(1, 18) < 1, p > .05$. In other words, injection of naloxone methiodide failed to alter the rate at which extinction accrued to the CS.

There were no differences between groups in levels of pre-CS freezing on test (Group Saline, $M = 1\%$ SEM = 1%; Group Naloxone, $M = 12\%$ SEM = 6%; Group Naloxone Methiodide $M = 15\%$ SEM = 8%), $F(1, 18) < 1.1, p > .05$. Collapsed across all groups, a within-subjects t test confirmed that there was a significant increase in freezing on the first CS presentation as compared with the pre-CS period (CS 1 freezing: Group Saline, $M = 25\%$ SEM = 5%; Group Naloxone $M = 55\%$ SEM = 5%; Group Naloxone Methiodide, $M = 27\%$ SEM = 8%; $p < .01$). There was a significant difference in levels of

freezing during CS presentations between rats in Group Naloxone versus rats in Groups Saline or Naloxone Methiodide, $F(1, 18) = 8.4, p = .01$. However, there was no significant difference in levels of freezing during CS presentations between rats in Group Saline versus rats in Group Naloxone Methiodide $F(1, 18) < 1, p > .05$.

Discussion

This experiment confirmed that sc injection of naloxone prior to nonreinforced presentations of an auditory CS previously paired with footshock impairs the development of the extinction that would otherwise accrue to that CS. This impairment was apparent during the six nonreinforced CS exposures under the influence of naloxone on Day 2 (extinction), as well as during the two nonreinforced exposures in the absence of the drug on Day 3 (test). In other words, the impairment of extinction by opioid receptor antagonism was observed in the presence and absence of the drug and therefore cannot be attributed to a nonspecific effect of the drug on freezing or locomotor activity. Moreover, this experiment shows that the impairment of extinction is mediated by opioid receptors in the CNS, because an injection of naloxone methiodide, a quaternary derivative of naloxone unable to cross the blood-brain barrier, failed to affect extinction. We consider it unlikely that the failure to detect any influence of peripheral opioid receptor antagonism on the extinction of fear can be attributed to the greater affinity for opioid receptors of naloxone compared with naloxone methiodide. First, the dose of naloxone methiodide (5 mg/kg) used was twice the dose of naloxone (2.5 mg/kg). Second, we have previously shown that this dose of naloxone methiodide is sufficient for the antagonism of peripheral opioid receptors (McNally, Johnston, & Westbrook, 2000).

Experiment 3

One interpretation of the effects of opioid receptor antagonists on the acquisition of aversive conditioning emphasizes that the release of opioid peptides during and immediately following a conditioning episode impairs memorial processing of that episode. Thus, opioid antagonists facilitate the acquisition of Pavlovian fear conditioning because they prevent this reactive inhibition on memory and thereby facilitate memory consolidation. An experimental design frequently used to provide evidence for this account involves postconditioning administration of opioid receptor antagonists. Therefore, the present experiment studied the effects of postextinction injections of naloxone on the development of fear extinction. Specifically, rats were allocated to four groups: Group Saline–Immediate, Group Naloxone–Immediate, Group Naloxone–30 min, and Group Naloxone–2 hr, in which the first group designation refers to the type of injection administered and the second group designation refers to the time that injection was administered following return to the home cage after the extinction session. If the effects of naloxone on extinction reported in Experiments 1 and 2 were due to the actions of the drug on some rehearsal or consolidation process occurring in the postextinction period, postextinction injections of naloxone should impair the development of extinction.

Method

Subjects and apparatus. The subjects were 32 experimentally naive, adult male Wistar rats (280–350 g) obtained from the same source and maintained under the same conditions as described previously. There were 8 rats per group. The apparatus used was that described previously in Experiment 2.

Drugs and procedure. Naloxone hydrochloride was dissolved in 0.9% (wt/vol) nonpyrogenic saline to obtain a concentration of 2.5 mg/ml. This 0.9% nonpyrogenic saline was used for control injections. All injections were given sc in the dorsal neck region in a volume of 1 ml/kg. The procedure for conditioning, extinction, and test was similar to that described for Experiment 2, with the exception that rats were injected with (a) saline immediately after extinction training, (b) naloxone immediately after extinction training, (c) naloxone 30 min after extinction training, or (d) naloxone 2 hr after extinction training.

Results

The mean ($\pm$ SEM) percentage of observations scored as freezing on test are shown in Figure 3. Inspection of the figure suggests that injection of naloxone at any of the time points following extinction training failed to modulate the development of extinction. There were no differences between groups in levels of pre-CS freezing, all $F_s(1, 28) < 1$, $p > .05$ (pre-CS freezing: Group Saline, $M = 9\%$ SEM = 7%; Group Naloxone–Immediate, $M = 8\%$ SEM = 4%; Group Naloxone–30 min, $M = 8\%$ SEM = 5%; Group Naloxone–2 hr, $M = 8\%$ SEM = 4%). Collapsed across groups, a within-subjects t test confirmed that there was a significant increase in freezing on the first CS presentation as compared with the pre-CS period (CS1 freezing: Group Saline, $M = 27\%$ SEM = 9%; Group Naloxone–Immediate, $M = 42\%$ SEM = 10%; Group Naloxone–30 min, $M = 45\%$ SEM = 10%; Group Naloxone–2 hr, $M = 40\%$ SEM = 7%; $p < .01$).

The analysis of freezing during CS presentations revealed no overall significant difference between rats injected with saline

Figure 3. Mean ($\pm$ SEM) percentage of observations scored as freezing on test in Experiment 3. Rats received an injection of 2.5 mg/kg naloxone or saline either immediately after extinction training, or an injection of naloxone 30-min or 2-hr after extinction training.

versus rats injected with naloxone, $F(1, 28) = 2.2$, $p > .05$. There was also no difference in levels of freezing between rats in Group Naloxone–Immediate versus rats in Groups Naloxone–30 min and Naloxone–2 hr, $F(1, 28) < 1$, $p > .05$. Finally, there was no difference in levels of freezing between rats in the latter two groups, $F(1, 28) < 1$, $p > .05$.

Discussion

This experiment showed that a postextinction injection of naloxone does not significantly alter the extinction that accrues to an auditory CS during nonreinforced presentations. These results contrast with the ability of preextinction injections of naloxone to impair that extinction. Therefore, the present results, when combined with those reported in Experiments 1 and 2, suggest that naloxone impairs the development of extinction only when it is present during nonreinforced CS exposures.

Experiment 4

The experiments reported here show that sc injection of naloxone impairs the extinction of fear conditioning. The aim of Experiment 4 was to determine whether sc injection of naloxone would also reverse the expression of any extinction that had already accrued to a CS as a consequence of nonreinforcement. The design was a 2×2 factorial, in which the first factor refers to the type of injection given prior to a single extinction session (naloxone vs. saline) and the second factor refers to the type of injection given prior to test (naloxone vs. saline). Injection of naloxone prior to extinction training should prevent extinction of fear to the CS. The question of interest here was whether an injection of naloxone prior to test would reinstate already extinguished fear (i.e., Group Saline–Naloxone).

Method

Subjects and apparatus. The subjects were 32 experimentally naive, adult male Wistar rats (280–350 g) obtained from the same source and

maintained under the same conditions as described previously. There were 8 rats per group (Group Saline–Saline, Group Saline–Naloxone, Group Naloxone–Saline, and Group Naloxone–Naloxone). The apparatus was the same as that described in Experiment 2.

Drugs and procedure. Naloxone hydrochloride was dissolved in 0.9% (wt/vol) nonpyrogenic saline to obtain a concentration of 2.5 mg/ml. This 0.9% nonpyrogenic saline was used for control injections. All injections were given sc in the dorsal neck region in a volume of 1 ml/kg. The procedure for conditioning and extinction was the same as that described for Experiment 2. The procedure for test was similar to that described in Experiment 2, with the exception that all rats were injected 20 min prior to test with either naloxone or saline according to their group allocation.

Results

The mean ($\pm$ SEM) percentage of observations scored as freezing during extinction and test are shown in the left and right panels, respectively, of Figure 4. Inspection of the left panel of Figure 4 confirms that an injection of naloxone prior to nonreinforced presentations of a CS impaired the extinction that would otherwise have accrued to that CS. Inspection of the right panel of Figure 4 suggests, first, that this impairment in extinction was observed independently of the presence of naloxone versus saline on test, and, second, that injection of naloxone on test did not reverse any extinction that might have already accrued to the CS. These observations were confirmed by the statistical analysis.

There were no differences between naloxone- and saline-treated rats in levels of pre-CS freezing during extinction, $F(1, 30) < 1$, $p > .05$. Collapsed across groups, a within subjects t test confirmed that there was significant increase in freezing on the first CS presentation as compared with the pre-CS period ($p < .01$). During extinction training, there was a significant overall difference in levels of freezing between rats injected with naloxone versus rats injected with saline, $F(1, 30) = 14.5$, $p < .01$. There was an overall linear decrease in freezing across the six nonreinforced presentations of the CS, averaged across groups, $F(1, 30) = 44.1$, $p < .01$. Moreover, this linear decrease in freezing interacted

with the differences between rats injected with naloxone versus rats injected with saline, $F(1, 18) = 15$, $p < .01$. In other words, the decrease in freezing across the extinction session was significantly greater among rats injected with saline as compared with rats injected with naloxone.

On test, there was a significant difference between groups in levels of pre-CS freezing such that rats that had been injected with naloxone prior to extinction displayed significantly lower pre-CS levels of freezing on test as compared with rats that had been injected with saline prior to extinction, $F(1, 28) = 4.9$, $p = .03$. There were no other differences between groups in pre-CS levels of freezing, $F_s(1, 28) < 1$, $p > .05$ (pre-CS freezing: Group Saline–Saline, $M = 55\%$ $SEM = 9\%$; Group Saline–Naloxone, $M = 53\%$ $SEM = 11\%$; Group Naloxone–Saline, $M = 35\%$ $SEM = 7\%$; Group Naloxone–Naloxone, $M = 34\%$, $SEM = 9\%$). Collapsed across groups, a within-subjects t test confirmed that there was significant increase in freezing on the first CS presentation as compared with the pre-CS period (CS1 freezing: Group Saline–Saline, $M = 68\%$ $SEM = 6\%$; Group Saline–Naloxone, $M = 59\%$ $SEM = 9\%$; Group Naloxone–Saline, $M = 83\%$ $SEM = 8\%$; Group Naloxone–Naloxone, $M = 88\%$, $SEM = 6\%$; $p < .0001$). The analysis of freezing during CS presentations revealed an overall significant main effect for injection of naloxone versus saline prior to extinction, $F(1, 28) = 28.4$, $p < .01$. However, there was no significant main effect for injection of naloxone versus saline prior to test, $F(1, 28) < 1$, $p > .05$. Finally, there was no significant interaction between injection of naloxone versus saline prior to extinction versus prior to test, $F(1, 28) < 1$, $p > .05$.

Discussion

This experiment confirmed that sc injection of naloxone impairs the extinction of Pavlovian fear conditioning, and showed that an injection of naloxone prior to test does not reinstate extinguished responding to an already extinguished CS. In other words, an injection of naloxone prior to nonreinforced presentations of a CS

Figure 4. Mean ($\pm$ SEM) percentage of observations scored as freezing during extinction training (left panel) and test (right panel) in Experiment 4. Rats received an injection of 2.5 mg/kg naloxone or saline prior to extinction training and an injection of 2.5 mg/kg naloxone or saline prior to test.

significantly impairs the extinction that would otherwise accrue to that CS, but these injections do not reverse any extinction that has already accrued to that CS. Together, these results show that opioid receptors regulate the development, but not the expression, of extinction of Pavlovian fear conditioning.

General Discussion

The experiments reported here used the freezing reactions of rats to examine the role of opioid receptors in the extinction of Pavlovian conditioned fear. Their results can be summarized as follows. Injection of naloxone prior to nonreinforced presentations of a previously conditioned auditory CS impaired the development of extinction, and this impairment was observed both within and between sessions. These effects of naloxone required the presence of the drug during the nonreinforced exposures, because injections of naloxone immediately, 30 min, or 2 hr after extinction were ineffective. Finally, a single injection of naloxone did not reinstate responding to an already extinguished CS. These results show for the first time that opioid receptors regulate the extinction of Pavlovian fear conditioning.

When combined with previous findings, the present experiments show that opioid receptors exert opposing influences on the acquisition and extinction of Pavlovian fear conditioning. Thus, naloxone enhances the acquisition (Fanselow, 1984; Fanselow & Bolles, 1979; Fanselow et al., 1980; Fanselow & Sigmundi, 1982; Lester & Fanselow, 1986; Young & Fanselow, 1992) and impairs the extinction of this fear, as indexed by freezing. This opposing role for opioid receptors in conditioning is notable for two reasons. First, many of the pharmacological manipulations that modulate extinction of Pavlovian fear conditioning have similar effects on the acquisition of that fear. For example, administrations of *N*-methyl-D-aspartate (NMDA) receptor antagonists impair the acquisition (Miserendino, Sananes, Melia, & Davis, 1990) as well as the extinction (e.g., Falls, Miserendino, & Davis, 1992; Santini, Muller, & Quirk, 2001) of fear conditioning, whereas NMDA receptor partial agonists facilitate this acquisition (Land & Riccio, 1999) and extinction (e.g., Ledgerwood, Cranney, & Richardson, 2003; Walker, Ressler, Lu, & Davis, 2002). Similar findings have been reported following interference with the signal transduction cascades subsequent to NMDA receptor activation (Lu, Walker, & Davis, 2001). Together, these results are usually interpreted with reference to the possibility that the extinction of Pavlovian fear conditioning does not involve the unlearning or erasure of an association, but instead the masking of the original CS-US association as a result of new learning. Thus, they are interpreted to mean that the neural mechanisms for this new learning are similar to the neural mechanisms for the original learning. At least one difficulty faced by this interpretation is that it fails to offer any neurobiological mechanism for distinguishing between the presence versus the absence of the US, and therefore fails to specify why extinction occurs. The present experiments have identified opioid receptors in the CNS as a candidate for such a differentiation between the presence and absence of the US.

This opposing role for opioid receptors in the acquisition and extinction of fear conditioning is also notable because current interpretations of opioid regulation of conditioning appear to struggle when pitted against the present data. Specifically, interpretations that emphasize a role for opioid peptides and their receptors

in regulating the sensory impact of the actual or expected US (Bolles & Fanselow, 1980; Fanselow, 1998) do not appear to explain the present data. These interpretations suppose that naloxone facilitates the acquisition of aversive conditioning because it increases the perceived intensity of the US. In the absence of a painful US (e.g., extinction) naloxone should not modulate learning. Similarly, interpretations that accord opioid peptides and their receptors a role in modulating memory for the conditioning episode also do not appear to explain the present data. These interpretations have argued that the release of opioid peptides during and immediately following a conditioning episode impairs the memorial processing of that episode (e.g., Gallagher, 1989). According to this line of reasoning, naloxone should have facilitated extinction by facilitating rehearsal or consolidation of the extinction memory, and postextinction injections of naloxone should have been just as effective as preextinction injections in preventing extinction. Neither prediction was confirmed in the present experiments.

These interpretations can be modified, however, to account for the present results. Rescorla (2001) followed earlier theorists (e.g., Amsel, 1958; Konorski, 1967; Wagner, 1981) by suggesting that the new learning that occurs during extinction is motivational or emotional in nature. Specifically, it is a motivational state or emotional response generated by detection of the discrepancy between the expected outcome (the US) and the actual outcome (no US). In the case of appetitive Pavlovian conditioning, the omission of the otherwise expected attractive US during extinction arouses an aversive motivational state or negative emotional response. Rescorla (2001) argued that the important consequence of arousal of this opponent motivational state is its association with any responses that might occur during extinction. In other words, this opponent motivational state counter-conditions or punishes the appetitive CR. In the case of the extinction of Pavlovian fear conditioning, this opponent motivational state could be identified as relief (Dickinson & Dearing, 1978; Konorski, 1967), which counterconditions species-typical defense responses such as freezing. Thus, the suggestion is that the actions of opioid peptides are critical for the extinction of fear conditioning because they mediate the relief occasioned by omission of the otherwise expected aversive US. Blockade of this action by naloxone impairs extinction because it prevents the relief that counter-conditions or otherwise inhibits the conditioned fear response (freezing). This interpretation of opioid function in Pavlovian conditioning, similar to that suggested by Schull (1979), explains the effects of opioid receptors agonists and antagonists on the acquisition as well as the extinction of fear conditioning. It is also very similar to the account of opioid function described by Fanselow (Bolles & Fanselow, 1980; Fanselow, 1998), with the difference that opioids are identified with modulation of the emotional, rather than sensory, processing of the footshock US. However, there is an important limitation to this possibility. Whereas there is evidence for an aversive emotional response accompanying the omission of an otherwise expected appetitive US (for reviews, see Daly, 1974; Wagner, 1969), there is currently no such evidence for relief or any other appetitive motivational state accompanying the omission of an otherwise expected aversive US during extinction.

In conclusion, the present experiments show that CNS opioid receptors mediate the extinction of Pavlovian fear conditioning. This role in extinction is opposite to the role of these receptors in

acquisition of such fear and could be interpreted with reference to roles in either appetitive motivation or relief. The precise anatomical locus and intracellular, as well as molecular, mechanisms for opioid receptor contributions to fear extinction remain to be determined.

References

- Amsel, A. (1958). The role of frustrative nonreward in noncontinuous reward situations. *Psychological Bulletin*, 55, 102–119.
- Bolles, R. C., & Fanselow, M. S. (1980). A perceptual-defensive-recuperative model of fear and pain. *Behavioral and Brain Sciences*, 3, 291–323.
- Bostock, E., Gallagher, M., & King, R. A. (1988). Effects of opioid microinjections into the medial septal area on spatial memory in rats. *Behavioral Neuroscience*, 102, 643–652.
- Carrasco, M. A., Dias, R. D., & Izquierdo, I. (1982). Naloxone reverses retrograde amnesia induced by electroconvulsive shock. *Behavioral and Neural Biology*, 34, 352–357.
- Collier, T. J., Quirk, G. J., & Routtenberg, A. (1987). Separable roles of hippocampal granule cells in forgetting and pyramidal cells in remembering spatial information. *Brain Research*, 409, 316–328.
- Daly, H. B. (1974). Reinforcing properties of escape from frustration aroused in various learning situations. In G. H. Bower (Ed.), *The psychology of learning and motivation* (Vol. 8, pp. 187–232). New York: Academic Press.
- Dickinson, A., & Dearing, M. F. (1978). Appetitive-aversive interactions and inhibitory processes. In A. Dickinson & R. A. Boakes (Eds.), *Mechanisms of learning and motivation: A memorial volume to Jerzy Konorski* (pp. 203–231). Hillsdale, NJ: Erlbaum.
- Falls, W. A., Miserendino, M. J. D., & Davis, M. (1992). Extinction of fear-potentiated startle: Blockade by an infusion of an NMDA receptor antagonist into the amygdala. *Journal of Neuroscience*, 12, 854–863.
- Fanselow, M. S. (1984). Opiate modulation of the active and inactive components of the postshock reaction: Parallels between naloxone pretreatment and shock intensity. *Behavioral Neuroscience*, 98, 269–277.
- Fanselow, M. S. (1998). Pavlovian conditioning, negative feedback, and blocking: Mechanisms that regulate association formation. *Neuron*, 20, 625–627.
- Fanselow, M. S., & Bolles, R. C. (1979). Naloxone and shock-elicited freezing in the rat. *Journal of Comparative and Physiological Psychology*, 93, 736–744.
- Fanselow, M. S., & Sigmundi, R. A., (1982). The enhancement and reduction of defensive fighting by naloxone pretreatment. *Physiological Psychology*, 10, 313–316.
- Fanselow, M. S., Sigmundi, R. A., & Bolles, R. C. (1980). Naloxone pretreatment enhances shock-elicited aggression. *Physiological Psychology*, 8, 369–371.
- Gallagher, M. (1989). Re-viewing modulation of learning and memory. In N. H. Weinberger & G. Lynch (Eds.), *Memory systems of the brain* (pp. 311–334). New York: Guilford Press.
- Gallagher, M., King, R. A., & Young, N. B. (1983, September 2). Opiate antagonists improve spatial memory. *Science*, 221, 975–976.
- Gallagher, M., Meagher, M. W., & Bostock, E. (1987). Effects of opiate manipulations on latent inhibition in rabbits: Sensitivity of the medial septal region to intracranial treatments. *Behavioral Neuroscience*, 101, 315–324.
- Hernandez, L. L., & Powell, D. A. (1980). Effects of naloxone on Pavlovian conditioning of eyeblink and heart rate responses in rabbits. *Life Sciences*, 27, 863–869.
- Hernandez, L. L., & Powell, D. A. (1983). Naloxone induces multiple effects on aversive Pavlovian conditioning. *Behavioral Neuroscience*, 97, 478–491.
- Hernandez, L. L., Watson, K. L., Fowler, B. M., Bair, K. D., & Singha, A. K. (1997). Opioid modulation of attention-related processes: Peripheral to central progression and development of mu influence as learning occurs. *Psychopharmacology*, 132, 50–60.
- Intorini, I. B., McGaugh, J. L., & Baratti, C. M. (1985). Pharmacological evidence of a central effect of naltrexone, morphine, and beta-endorphin and a peripheral effect of met- and leu-enkephalin on retention of an inhibitory response in mice. *Behavioral and Neural Biology*, 44, 434–446.
- Intorini-Collison, I. B., Nagahara, A. H., & McGaugh J. L. (1989). Memory enhancement with intra-amygdala posttraining naloxone is blocked by concurrent administration of propranolol. *Brain Research*, 476, 94–101.
- Kameyama, T., Nabeshima, T., & Kozawa, T. (1986). The antagonistic effects of naloxone on cycloheximide and anisomycin-induced amnesia. *Pharmacology Biochemistry and Behavior*, 25, 567–572.
- Konorski, J. (1967). *The integrative activity of the brain*. Chicago: University of Chicago Press.
- Land, C., & Riccio, D. C. (1999). D-Cycloserine: Effects on long-term retention of a conditioned response and on memory for contextual attributes. *Neurobiology of Learning and Memory*, 72, 158–168.
- Ledgerwood, L., Cranney, J. C., & Richardson, R. (2003). Effects of D-cycloserine on extinction of conditioned freezing. *Behavioral Neuroscience*, 117, 341–349.
- Lester, L. S., & Fanselow, M. S. (1986). Naloxone's enhancement of freezing: Modulation of perceived intensity or memory processes? *Physiological Psychology*, 14, 5–10.
- Liang, K. C., Messing, R. B., & McGaugh, J. L. (1983). Naloxone attenuates amnesia caused by amygdaloid stimulation: The involvement of a central opioid system. *Brain Research*, 271, 41–49.
- Lu, K. T., Walker, D. L., & Davis M. (2001). Mitogen-activated protein kinase cascade in the basolateral nucleus of amygdala is involved in extinction of fear-potentiated startle. *Journal of Neuroscience*, 21, RC162.
- McGaugh, J. L., Intorini-Collison, J. B., & Nagahara, A. H. (1988). Memory-enhancing effects of posttraining naloxone: Involvement of beta-noradrenergic influences in the amygdaloid complex. *Brain Research*, 446, 37–49.
- McNally, G. P., & Akil, H. (2002). Opioid peptides and their receptors. In K. Davis, D. Charney, J. T. Coyle, & C. Nemeroff, (Eds.), *Neuropsychopharmacology: Fifth generation of progress* (pp. 35–46). New York: Lippincott, Williams & Wilkins.
- McNally, G. P., Johnston, I. N., & Westbrook, R. F. (2000). A peripheral, intracerebral, or intrathecal administration of an opioid receptor antagonist blocks illness-induced hyperalgesia in the rat. *Behavioral Neuroscience*, 114, 1183–1190.
- Miserendino, M. J., Sananes, C. B., Melia, K. R., & Davis M. (1990, June 21). Blocking of acquisition but not expression of conditioned fear-potentiated startle by NMDA antagonists in the amygdala. *Nature*, 345, 716–718.
- Nabeshima, T., Kozawa, T., Furukawa, H., & Kameyama, T. (1986). Phencyclidine-induced retrograde amnesia in mice. *Psychopharmacology (Berlin)*, 89, 334–337.
- O'Brien, R. G., & Kaiser, M. K. (1985). MANOVA method for analyzing repeated measures designs: An extensive primer. *Psychological Bulletin*, 97, 316–333.
- Rescorla, R. A. (2001). Experimental extinction. In R. R. Mowrer & S. B. Klein (Eds.), *Handbook of contemporary learning theories* (pp. 119–154). Hillsdale, NJ: Erlbaum.
- Rudy, J. W., Kuwagama, K., & Pugh, C. R. (1999). Isolation reduces contextual but not auditory-cue fear conditioning: A role for endogenous opioids. *Behavioral Neuroscience*, 113, 316–323.
- Rush, D. K. (1986). Reversal of scopolamine-induced amnesia of passive

- avoidance by pre- and post-training naloxone. *Psychopharmacology (Berlin)*, 89, 296–300.
- Santini, E., Muller, R. U., & Quirk, G. J. (2001). Consolidation of extinction learning involves transfer from NMDA-independent to NMDA-dependent memory. *Journal of Neuroscience*, 21, 9009–9017.
- Schull, J. (1979). A conditioned opponent theory of Pavlovian conditioning and habituation. In G. H. Bower (Ed.), *The psychology of learning and motivation* (Vol. 13, pp. 57–90). New York: Academic Press.
- Vigorito, M., & Ayres, J. J. B. (1987). Effect of naloxone on conditioned suppression in rats. *Behavioral Neuroscience*, 101, 576–586.
- Wagner, A. R. (1969). Frustrative nonreward: A variety of punishment. In B. A. Campbell & R. M. Church (Eds.), *Punishment and aversive behavior* (pp. 157–181). New York: Appleton-Century-Crofts.
- Wagner, A. R. (1981). SOP: A model of automatic memory processing in animal behavior. In N. E. Spear & R. R. Miller (Eds.), *Information processing in animals: Memory mechanisms* (pp. 5–47). Hillsdale, NJ: Erlbaum.
- Walker, D. L., Ressler, K. J., Lu, K. T., & Davis M. (2002). Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of D-cycloserine as assessed with fear-potentiated startle in rats. *Journal of Neuroscience*, 22, 2343–2351.
- Young, S. L., & Fanselow, M. S. (1992). Associative regulation of Pavlovian fear conditioning: Unconditional stimulus intensities, incentive shifts, and latent inhibition. *Journal of Experimental Psychology: Animal Behavior Processes*, 18, 400–413.

Received February 4, 2003

Revision received May 15, 2003

Accepted June 3, 2003 ■