Temporally Graded, Context-Specific Retrograde Amnesia and Its Alleviation by Context Preexposure: Effects of Postconditioning Exposures to Morphine in the Rat

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Five experiments studied retrograde impairments in Pavlovian fear conditioning following prolonged exposure to the opioid receptor agonist morphine. Injections of morphine commencing 1–7 days but not 14 days after conditioning produced amnesia for that conditioning episode. This amnesia was (a) selective such that morphine impaired freezing to the conditioning context but not to the auditory conditioned stimulus, (b) independent of the interval between the last injection of morphine and test, and (c) accompanied by a failure of contextual discrimination. Context preexposure protected context conditioning and discrimination from the amnesic effects of morphine. These results show that retrograde deficits in contextual fear conditioning are mediated by failures to consolidate a contextual representation.

Placed in a novel context (a distinctive chamber) and exposed to brief but aversive footshocks, rats learn about this relation between the context and the footshock unconditioned stimulus (US). They exhibit this learning when reexposed to the context in a diverse but correlated range of behavioral and autonomic reactions that include freezing, hypoalgesia, potentiated startle, and increased arterial blood pressure (for reviews, see Davis, 1992; Fanselow, 2000; LeDoux, 1993). The contents of this learning are commonly viewed in terms of the formation of excitatory associations between a representation of the context and a defensive or fear motivational system aroused by the aversive US (e.g., Konorski, 1967; but see Bolles & Fanselow, 1980). The consequence of these associations is that reexposure to the context provokes the various reactions (freezing, etc.) appropriate to arousal of the fear system. An implication of this view is that the levels of these conditional fear reactions will be influenced by the nature of the context representation that entered into excitatory associations with the fear system aroused by the US. More specifically, fear reactions will be increased among rats shocked in a context whose various cues (its appearance, smell, texture, shape, etc.) have been combined into a unitary representation in comparison with the performances of rats that have not developed such a representation.

There is considerable evidence for this proposal concerning the relation between context learning and context fear conditioning. For instance, the levels of freezing elicited by reexposure to a

shocked context are positively related to the time spent in the context before the occurrence of footshock. Specifically, rats shocked 120 s after placement in a novel context freeze more when reexposed to that context than rats shocked either immediately or shortly (e.g., 10 s) after placement in that context (Kiernan & Westbrook, 1993; but see Bevins, McPhee, Rahut, & Ayres, 1997). Further, this function relating freezing to the placement–footshock interval is shifted upward among rats preexposed to that context. In other words, rats that have already learned about the context as a result of preexposure develop better conditioned performance at each of the placement–footshock intervals than rats exposed to these intervals in a novel context (Fanselow, 1990; Kiernan & Westbrook, 1993). This facilitatory effect on conditioned performance depends on rats being preexposed to the conditioning context (i.e., to a set of features in particular relations to each other), because preexposure to its features in different relations to each other failed to facilitate context-controlled freezing (Rudy & O'Reilly, 1999). Finally, rats preexposed to a context and then shocked 120 s after exposure to that context generalized freezing to a second context less than rats shocked after this delay in a novel context who, in turn, generalized freezing less than rats shocked 10 s after exposure to a novel context (Kiernan & Westbrook, 1993; Westbrook, Good, & Kiernan, 1994).

These findings have been taken to mean that context fear conditioning involves two distinct sets of processes. The first records the spatial–temporal conjunctions among the several cues comprising the context and combines these into a unitary representation (Fanselow, 1990; McLaren, Kaye, & Mackintosh, 1989; McLaren & Mackintosh, 2000; O'Reilly & Rudy, 2001). The second set of processes forms excitatory associations between the output of this configural system and the fear motivational system. The formation of this unitary representation and its association with the fear system is necessary but not sufficient for that context to subsequently control substantial levels of freezing. An additional requirement for such control is that the unitary representation of the shocked context be rehearsed, consolidated, or other-

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wise rendered relatively permanent. Evidence for this additional requirement can be derived from two sources. First, Rudy and colleagues (e.g., Pugh et al., 1998, 2000; Rudy, 1996) exposed rats to conditioned stimulus (CS)–footshock pairings in a distinctive context and then subjected them to either social isolation or a single intraperitoneal injection of an opioid agonist (morphine) or of the bacterial endotoxin lipopolysaccharide (LPS). These rats subsequently exhibited just as much freezing as did control rats when tested with the CS but showed reductions in the levels of freezing when reexposed to the context where the pairings had occurred. Moreover, these reductions in context-controlled freezing depended on the manipulations (social isolation, injection with morphine or LPS) being performed shortly (a few minutes to an hour) after the fear conditioning episode: Rats treated in any of these ways some time after that episode exhibited just as much freezing to both the CS and the context as did control rats. Second, Fanselow and colleagues (e.g., Anagnostaras, Maren, & Fanselow, 1999; Kim & Fanselow, 1992; Maren, Aharonov, & Fanselow, 1997) also exposed rats to CS–footshock pairings in a distinctive context but then subjected them to hippocampal lesions. The hippocampally damaged rats subsequently exhibited just as much freezing as did control rats when tested with the CS but showed reductions in the levels of freezing when reexposed to the context where the pairings had occurred. These reductions in context-controlled freezing also depended on the lesion being performed shortly (1–21 days) after the fear conditioning episode: Rats subjected to hippocampal lesions some time (28–100 days) after that episode exhibited just as much freezing to both the CS and the context as control rats.

The amnesic effects produced by these manipulations appear to be due to a disruption of the processes involved in consolidation of the configural representation of the context rather than of those that associate this representation with the fear motivation system. More specifically, these effects appear to be specific to a disruption of the processes involved in consolidation of the configural representation of a novel context. Rudy and colleagues reported that the amnesic effect of social isolation or morphine was removed among rats that had already learned about the context as a result of preexposure (Rudy, 1996; Rudy, Kuwagama, & Pugh, 1999). They found that rats subjected to social isolation or morphine after a shocked exposure to a context showed just as much freezing as did control rats if the former had been preexposed to the context. Moreover, rats injected with morphine after context preexposure did not show the facilitation of freezing observed when rats are shocked in a preexposed context (e.g., Fanselow, 1990; Kiernan & Westbrook, 1993). Thus, morphine injected shortly after preexposure disrupted consolidation of the configural representation taken to mediate the increased levels of freezing when rats are shocked in the preexposed context. Likewise, Fanselow and colleagues reported that the retrograde amnesia for a shocked context among rats subjected to hippocampal lesions was abolished if rats had already learned about the context as a result of preexposure (Anagnostaras, Gale, & Fanselow, 2001; Young, Bohenek, & Fanselow, 1994).

The common impairment of context learning and/or context fear conditioning by these diverse postconditioning manipulations (social isolation, injection of morphine or LPS, hippocampal lesion) does not necessarily imply that this outcome is produced by a common mechanism. Nevertheless, the endogenous opioids may

constitute just such a common mechanism. There is compelling evidence that the actions of these peptides play a causal role in many retrograde amnesic syndromes. For example, the retrograde amnesic effects of social isolation are prevented by administrations of a μ -opioid receptor (MOR) antagonist and (as noted previously) mimicked by a single injection of morphine (Rudy et al., 1999). The amnesic effects produced by hippocampal stimulation (Collier, Quirk, & Routtenberg, 1987), amygdala stimulation (Liang, Messing, & McGaugh, 1983), electroconvulsive footshock (Carrasco, Dias, & Izquierdo, 1982), protein synthesis inhibitors (Kameyama, Nabeshima, & Kozawa, 1986), phencyclidine (Nabeshima, Kozawa, Furukawa, & Kameyama, 1986), and scopolamine (Rush, 1986) are also prevented by MOR antagonism. Moreover, we have shown that the opioid peptides are important mediators of the effects of immune activation (McNally, Johnston, & Westbrook, 2000) and thus may mediate the ability of that activation (e.g., injection of the HIV-1 protein coat or LPS) to impair contextual learning (Pugh et al., 2000).

This evidence for a causal involvement of opioid peptides in retrograde amnesia has been derived almost exclusively from studies using single exposures to agonists or antagonists in the minutes to hours following conditioning. However, recent neurobiological findings suggest that similar amnesic effects should be observed, and indeed may be more pronounced, if exposures to MOR agonists occur in the days to weeks following conditioning. For example, Eisch, Barrot, Schad, Self, and Nestler (2000) reported that prolonged exposures to an MOR agonist reduced the number and survival of proliferating cells in the rat hippocampus. Neurogenesis is frequently invoked as an important mechanism for hippocampal-dependent learning and memory. Hence, inhibition of neurogenesis by prolonged opiate exposures suggests that hippocampal-dependent learning and memory may be impaired by such exposures. Similarly, Pu, Bao, Xu, Ma, and Pei (2002) reported that prolonged exposures to an MOR agonist produced severe impairments in hippocampal function, as indexed by alterations in both long-term potentiation (another putative physiological mechanism for learning and memory) and spatial learning in the water maze (Morris, 1981). These findings suggest that activation of opioid receptors in the days to weeks following fear conditioning should interfere with the hippocampal-dependent consolidation, rehearsal, or otherwise permanent rendering of a unified contextual representation, thereby producing retrograde deficits in contextual fear conditioning.

The general aim of the present series of experiments was to study this suggestion concerning the amnesic effects of postconditioning exposures to the MOR agonist morphine (McNally & Akil, 2002). In each experiment, rats were exposed to a single auditory CS (clicker)–US (footshock) pairing in a distinctive context. Then they received daily injections of morphine or saline commencing at varying intervals (1–21 days) after this fear-conditioning episode. Finally, the fear reactions (freezing) elicited by reexposure to the conditioning context and to the CS were assessed at varying intervals (1–21 days) following the last injection. Experiment 1 studied whether postconditioning exposures to morphine would spare freezing to the CS paired with footshock but impair freezing to the context where the pairing occurred. Experiment 1 also studied whether this impairment in context-controlled freezing was a function of the interval between conditioning and morphine exposures. This was done to determine whether post-

conditioning morphine exposures impaired recent but not remote memories for the dangerous context. Experiments 2 and 3 studied whether this impairment in context-controlled freezing would recover across an interval between the termination of morphine exposures and test. Experiment 4 studied whether preexposures to the conditioning context would alleviate impairments in context-controlled freezing. Finally, Experiment 5 studied (a) whether the impairment in freezing to the previously shocked context was accompanied by impairments in discrimination between that and a second, different context and (b) whether preexposures to the conditioning context would alleviate this impairment in context discrimination.

Experiment 1

Rudy (1996) reported that rats subjected to 3 hr of social isolation shortly after two CS–footshock pairings subsequently exhibited reduced levels of freezing when reexposed to the context where the CS–footshock pairings had occurred but did not differ from control rats in their levels of freezing when tested with the CS. This retrograde amnesia was mediated by activity at opioid receptors because it could be prevented by administrations of an MOR antagonist (Rudy et al., 1999). Kim and Fanselow (1992) exposed rats to 15 CS–footshock pairings and then subjected the rats to lesions of the dorsal hippocampus 1, 7, 14, or 28 days later. They reported that such lesions spared freezing to the CS but reduced freezing to the conditioning context. Moreover, this reduction in freezing was temporally graded such that rats subjected to lesions 1 or 7 days after conditioning froze less than rats whose hippocampus was destroyed 28 days after conditioning. Similar findings, but with different temporal gradients, were reported by both Maren et al. (1997) and Anagnostaras et al. (1999).

Experiment 1 had two aims. The first was to determine whether activation of the MOR, identified by Rudy et al. (1999) as critical for the retrograde amnesia produced by various manipulations in the hours following conditioning, would produce retrograde amnesia when this activation occurred in the days to weeks following conditioning. The second aim was to determine whether this retrograde amnesia displayed a temporal gradient such that recent but not remote conditioning episodes were impaired by postconditioning exposures to morphine. Rats were exposed to a single auditory CS (clicker)–footshock pairing in a distinctive context. For control rats, injections of saline commenced either 1 day or 14 days after this conditioning episode and were repeated daily for 14 days. For experimental rats, injections of morphine commenced either 1 day, 3 days, 7 days, or 14 days after this conditioning episode and were repeated daily for 14 days. All rats were tested for their fear reactions (freezing) to the context where conditioning occurred on the day following the last injection and for freezing to the CS on the day following context test.

Method

Subjects. The subjects were 44 experimentally naive, adult, male Wistar rats (280–350 g) obtained from a commercial supplier (Gore Hill Research Laboratories, Sydney, Australia). After arrival, the rats were housed in groups of 8 in plastic boxes (67-cm length $\times$ 30-cm width $\times$ 22-cm height) with food and water continuously available. The boxes were kept in an air-conditioned colony room maintained on a 12:12-hr light–dark cycle (lights on at 7:00 a.m.). Each rat was handled 10–30 s per day

across 5 days before the start of the experiment and on each day of the experiment.

Apparatus. Conditioning and CS testing were conducted in different chambers. Four identical chambers (20-cm height $\times$ 21-cm length $\times$ 23-cm width) were used to shock the rats and to test for contextually controlled freezing. The front and rear walls of these chambers as well as the hinged lid were constructed of 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 0.5-s, 0.8-mA unscrambled AC 50-Hz footshock from a constant-current generator that was delivered to the floor of each chamber. The current available to each floor could be adjusted using an in-line milliamperemeter. Each chamber stood 5 cm above a tray of paper pellet bedding (Fibrecycle, Mudgeeraba, Australia) that was changed between rats. These four chambers were located within separate compartments of a wooden cabinet. The door of each compartment was kept open to permit observation of the rat. A second set of two plastic chambers (16-cm height $\times$ 40-cm length $\times$ 26-cm width) was used to test performance to the auditory CS. The front of each of these chambers was constructed of Perspex. The floor, side walls, and rear walls were made of plastic, and the roof was made of stainless steel rods. These chambers were located on the roof of the wooden cabinet that contained the footshock chambers. The discrete auditory CS consisted of a 74-dB clicker (10-Hz spike; rise time $< 10 \mu\text{s}$, decay time = 250 μs) delivered from a speaker located in the ceiling of the experimental room. The background noise in the room was 69 dB. The stimulus and background intensities were measured with a sound level meter (A scale; Type 2235, Brüel-Kjaer Instruments, Marlborough, MA) whose microphone was placed in the center of each chamber.

Procedure. The experiment involved three phases: fear conditioning, injections of morphine or saline, and testing. For fear conditioning, rats were transported in squads of 4 and placed in the conditioning chambers. One minute and 50 s later the 10-s CS was presented, which coterminated with delivery of footshock. Rats remained in the chambers for a further 30 s before being returned to their home cage. All rats were subjected to fear conditioning on the same day.

All injections were subcutaneous in the dorsal neck region and were given in a volume of 1 ml/kg, once daily. For morphine injections, an ascending regime, previously shown to induce analgesic tolerance (Westbrook, Greeley, Nabke, & Swinbourne, 1991), was used. Rats were injected with 2.5 mg/kg morphine for 3 days, 5.0 mg/kg morphine for 3 days, 10.0 mg/kg morphine for 3 days, and finally 20.0 mg/kg morphine for 5 days. For saline injections, rats were injected with 0.9% (wt/vol) sterile saline. Saline injections commenced either 1 day ($n = 8$) or 14 days ($n = 6$) following the conditioning episode. Morphine injections commenced 1 day ($n = 8$), 3 days ($n = 8$), 7 days ($n = 8$), or 14 days ($n = 6$) following the conditioning episode.

All rats were tested for context freezing the day following the last injection and were tested for CS freezing the day following context test. For context testing, rats were placed in the conditioning chamber, and their behavior was observed for 8 min. For CS testing, rats were placed in the second set of chambers for 2 min, and their behavior was observed. The CS was then presented for 3 min, and their behavior was observed. The duration of context and CS tests differed because pilot studies indicated that the levels of contextual- and CS-controlled freezing would be similar across these durations.

An additional two groups of rats ($n = 8$) were included at the conclusion of the experiment. These rats were subjected to the same conditioning arrangement as described previously. The day after conditioning, rats were given a single injection of morphine (2.5 mg/kg) or saline. These rats were tested for their fear reactions to the context the day following injection and for their fear reactions to the CS the day following context test. These two groups were included to verify that a single exposure to morphine 1 day following conditioning was unable to produce retrograde amnesia for that conditioning.

Scoring and statistics. In this and subsequent experiments, performance to the context and CS on test was videotaped, and the levels of freezing were measured with a time-sampling procedure in which the rat's behavior was scored as freezing or not freezing every 2 s. Freezing was defined as the absence of all movement except those related to breathing (Fanselow, 1990). The percentage of all samples scored as freezing was determined for each rat. Two observers, one of whom was unaware of the rat's treatment condition, scored the videotape of each rat. The unaware observer scored the rats behavior at variable time sampling intervals across the experiments (i.e., once every 2 s, 4 s, or 5 s). Thus, the scores from the aware observer were used for analysis. The agreement between these observer ratings (i.e., the correlation between the percentages of observations scored as freezing per rat) were very high and consistently exceeded 0.9. The data were analyzed by means of a planned orthogonal contrast testing procedure in which context and CS tests were treated as repeated measures. The Type I error rate was controlled at 0.05 for each contrast tested using the method described by Hays (1972).

Results

The left panel of Figure 1 shows the mean and the standard error of the mean (*SEM*) levels of freezing during the context test conducted the day after the last injection of morphine or saline. Inspection of the panel indicates that the levels of freezing among rats conditioned either 1 day or 14 days prior to saline injections did not differ. In contrast, freezing among rats conditioned 1, 3, 7, or 14 days prior to morphine injections did differ such that freezing increased as the interval between conditioning and the start of morphine injections increased. Freezing during the CS test conducted the day after context test is shown in the right panel of Figure 1. Levels of freezing in the new chamber during the 2-min preceding presentation of the CS were low (1-day mean for saline = 23%, *SEM* = 9%; 14-day mean for saline = 18%, *SEM* = 4%; 1-day mean for morphine = 3%, *SEM* = 1%; 3-day mean for morphine = 3%, *SEM* = 1%; 7-day mean for morphine = 17%, *SEM* = 5%; 14-day mean for morphine = 15%, *SEM* = 3%). Freezing to the CS among rats conditioned either 1 day or 14 days prior to 14 days of saline injections did not differ. Freezing to the

CS among rats conditioned 1, 3, 7, or 14 days prior to morphine injections was variable and did not appear to reliably differ from rats receiving saline injections.

These observations were confirmed by the statistical analysis. There was no main effect for injection type (morphine vs. saline), $F(1, 38) = 2.5, p > .05$. There was no main effect for the interval between conditioning and the start of injections, as indexed by linear trend, among morphine-treated rats, $F(1, 38) = 1.0, p > .05$. There was also no main effect for test type (context vs. CS), $F(1, 38) < 1, p > .05$.

There was a significant interaction for the influence of injection type (morphine vs. saline) and test type (context vs. CS), $F(1, 38) = 4.2, p < .05$. Moreover, for morphine-treated rats, there was a significant interaction of the influence of the interval between conditioning and the start of injections and test type (context vs. CS), $F(1, 38) = 6.6, p < .05$. From inspection, these interactions indicate that (a) the history of morphine injections impaired conditioning to the context but not to the CS and (b) this selective impairment in context freezing was a linear function of the interval between conditioning and the start of injections.

Discussion

This experiment has revealed that postconditioning exposures to morphine spare freezing to a CS paired with a footshock US but impair freezing to the context where this pairing occurred. Further, this impairment was temporally graded such that rats subjected to morphine exposures 14 days after fear conditioning exhibited more freezing when reexposed to the previously shocked context than rats subjected to morphine exposures 1, 3, or 7 days after fear conditioning. Thus, postconditioning exposures to morphine appear to disrupt recent but not remote memories of a dangerous context. This retrograde amnesia for contextual fear memory was dependent on rats being subjected to repeated morphine exposures because an amnesic effect was not observed if rats received a single 2.5 mg/kg injection of morphine the day after conditioning. When tested in the previously shocked context the day after that injection, these rats displayed freezing on 57% of observations (*SEM* = 5). Rats exposed to saline the day after conditioning and tested the day after that exposure displayed freezing on 52% of occasions (*SEM* = 7). There was no significant difference between these groups, $F(1, 14) < 1, p > .05$.

Experiment 2

The results of Experiment 1 show that repeated injections of morphine produce a temporal gradient of retrograde amnesia for contextual but not auditory CS fear conditioning. These results with morphine exposures parallel those obtained with postconditioning hippocampal lesions: In both cases, context- but not CS-controlled freezing is impaired, and this impairment is negatively related to the interval (days–weeks) between conditioning and either morphine exposures or hippocampal lesions (e.g., Anagnostaras et al., 1999; Kim & Fanselow, 1992; Maren et al., 1997). Hence, the present results could be interpreted to mean that the history of morphine exposures impaired a hippocampal-dependent consolidation of contextual memories. However, an alternate interpretation of these results is that the history of morphine exposures simply impaired retrieval of the relevant contextual informa-

Figure 1. Mean and *SEM* levels of freezing on context test (left panel) and CS test (right panel) in Experiment 1. Rats were subject to a single CS–footshock pairing in a distinctive context. They were then injected with morphine or saline every day for 14 days, commencing at varying intervals following this conditioning. Finally, rats were tested for freezing to the context the day after the last injection and for freezing to the CS the day after the context test. CS = conditioned stimulus.

tion on test (e.g., Bouton, 1993; Weiskrantz, 1966). For example, state-dependent processes, such as those produced by the presence of either morphine and/or morphine withdrawal, could have impaired retrieval of the conditioning memory on test. The temporal gradient of retrograde amnesia could then be explained by supposing that the nature of the retrieval cues used varies as a function of time since conditioning. If the retrograde deficit detected in Experiment 1 reflects a retrieval failure, then it might be affected by the passage of time. Specifically, this deficit could be alleviated by interpolation of an interval between injections of morphine and test (Bouton, 1993; Weiskrantz, 1966). Indeed, there is some evidence that the retrograde amnesia induced by postconditioning manipulations of hippocampal function is lost across a retention interval (Hughes, 1969).

The aim of Experiment 2 was to examine these contrasting explanations of the results found in the previous experiment. The design was a 2×3 factorial. The first factor refers to the type of injection (morphine vs. saline) that was started the day following conditioning and repeated every day for 2 weeks. The second factor refers to the length of the interval that was interpolated between the last of these injections and the context test (1, 7, or 21 days). Rats were tested for their fear reactions to the auditory CS the day after context test. If the deficit found in the previous experiment was due to the disruption of a consolidation process by postconditioning injections of morphine, this deficit should be independent of the interval between the cessation of morphine injections and test; in contrast, if the deficit was due to variations in retrieval, the deficit should be observed among rats tested shortly but not some time after cessation of morphine injections.

Method

Subjects and apparatus. The subjects were 48 experimentally naive rats (280–350 g) of the same sex and strain and obtained from the same source as in Experiment 1. They were kept under the conditions reported previously. The apparatus was that used in Experiment 1.

Procedure. The experiment involved three phases: fear conditioning, morphine or saline injections, and testing. Fear conditioning was identical to that described in Experiment 1. All rats were subjected to fear conditioning on the same day. Morphine exposures began the day following fear conditioning. Rats received daily injections of morphine ($n = 24$) or saline ($n = 24$) in the manner described in Experiment 1. Rats were tested for context freezing 1, 7, or 21 days following the last injection. The test for CS-elicited freezing took place the day after context test (i.e., 2, 8, or 22 days following the last injection). One rat injected with morphine died during the course of the experiment. Thus, only 7 rats were tested at the 7-day retention interval.

Results

The mean and *SEM* levels of freezing during context test are shown in the left panel, and the mean and *SEM* levels of freezing during CS test are shown in the right panel of Figure 2. Levels of freezing in the new chamber during the 2-min period preceding presentation of the CS were low (1-day mean for saline = 14%, *SEM* = 4%; 1-day mean for morphine = 5%, *SEM* = 2%; 7-day mean for saline = 6%, *SEM* = 4%; 7-day mean for morphine = 2%, *SEM* = 1%; 21-day mean for saline = 6%, *SEM* = 2%; 21-day mean for morphine = 6%, *SEM* = 1%). From inspection of Figure 2, it is apparent that morphine-treated rats displayed less

Figure 2. Mean and *SEM* levels of freezing on context test (left panel) and CS test (right panel) in Experiment 2. Rats were subject to a single CS–footshock pairing in a distinctive context. They were then injected with morphine or saline every day for 14 days, commencing the day following conditioning. Finally, rats were tested for freezing to the context 1, 7, or 21 days after the last injection and for freezing to the CS the day after the context test. CS = conditioned stimulus.

freezing than saline-treated animals when tested in the conditioning context but not when tested with the CS and that this impairment was not alleviated when an interval was interpolated between the last injection and test.

The analysis confirmed these observations. There was an overall main effect for injection (morphine vs. saline), $F(1, 41) = 8.4$, $p < .05$. There was no main effect for retention interval, as measured by linear trend, $F(1, 41) < 1$, $p > .05$. There was also no overall 2×3 interaction, $F(1, 41) < 1$, $p > .05$. There was no overall difference in the levels of freezing observed on context and CS tests, $F(1, 41) = 1.7$, $p > .05$.

There was an interaction between the effect of injection type (morphine vs. saline) and test type (context vs. CS) such that morphine selectively impaired freezing on context test, $F(1, 41) = 9.2$, $p < .05$. There was no interaction between retention interval and test type, $F(1, 41) < 1$, $p > .05$. Finally there was no $2 \times 3 (\times 2)$ interaction, $F(1, 41) < 1$, $p > .05$.

Discussion

This experiment has confirmed the results of Experiment 1 and shown that prolonged exposure to morphine is associated with a retrograde deficit specific to contextual fear conditioning. It is important to note that this deficit was not alleviated by interpolation of a retention interval between the last drug exposure and test. This finding suggests that postconditioning exposures to morphine result in relatively permanent impairments of context-controlled freezing. This suggestion is consistent with the hypothesis that postconditioning exposures to morphine disrupt the consolidation rather than the retrieval of context fear conditioning.

Experiment 3

Experiment 1 studied the temporal gradient of retrograde deficits in fear memories produced by repeated exposures to morphine. There was such a gradient. Experiment 2 studied whether those

memories recovered across a retention interval between the termination of morphine exposure and test. They did not. However, the evidence that morphine exposures produced a temporally graded retrograde deficit for contextual fear memories independently of when those memories were assessed relies on a comparison between those experiments. Therefore, we sought to provide a within-experiment replication of these results. In Experiment 3 we varied the interval between conditioning and the start of morphine injections as well as the interval between cessation of these injections and test. We did this to confirm that the temporal gradient of retrograde deficits identified in Experiment 1 was independent of when rats were tested, as may have been shown in Experiment 2. The design was a 2×2 factorial. The first factor was when rats were conditioned relative to the first injection of morphine (1 day or 21 days). The second factor was when rats were tested for their fear reactions to the context relative to the last of these injections of morphine (1 day or 21 days). CS test was conducted the day after context test. The four groups are designated 1:1, 1:21, 21:1, and 21:21, where the first number refers to the interval (1 day or 21 days) between conditioning and start of morphine injections, and the second number refers to the interval (1 day or 21 days) between the final morphine injection and test.

Method

Subjects and apparatus. The subjects were 32 experimentally naive rats (280–350 g) of the same sex and strain and from the same source as in Experiment 1. They were kept under the same conditions described previously. The apparatus was that used in Experiment 1.

Procedure. The experiment involved three phases: fear conditioning, morphine injections, and testing. All rats were subjected to fear conditioning on the same day. Fear conditioning was identical to that used in Experiment 1. Morphine injections began either 1 day ($n = 16$) or 21 days ($n = 16$) following fear conditioning. Rats received daily injections of morphine for 2 weeks, as described previously. Rats were tested for context freezing either 1 day ($n = 16$) or 21 days ($n = 16$) following the last morphine injection. The test for CS-elicited freezing took place the day after context test (i.e., 2 days or 22 days following the last injection). Three rats died across the course of the experiment. Thus, there were 7 rats per group in Groups 1:1, 21:1, and 21:21, and 8 rats in Group 1:21.

Results

The mean and *SEM* levels of freezing are shown for the context test in the left panel of Figure 3, and those for the CS test are shown in the right panel of Figure 3. Levels of freezing in the new chamber during the 2-min period preceding presentation of the CS were low (Group 1:1 mean = 16%, *SEM* = 5%; Group 1:21 mean = 8%, *SEM* = 4%; Group 21:1 mean = 8%, *SEM* = 2%; Group 21:21 mean = 10%, *SEM* = 3%). Inspection of Figure 3 indicates that rats conditioned 1 day prior to the start of morphine injections displayed less freezing on context test than rats conditioned 21 days prior to morphine injections and that this retrograde amnesia was not influenced by the interval (1 or 21 days) between the final morphine injection and test. In contrast, neither of these intervals affected freezing to the CS.

These observations were confirmed by the statistical analysis. There was no main effect of the interval between conditioning and the start of morphine injections, $F(1, 25) = 1.9, p > .05$. There was no main effect for the interval between the last of these

Figure 3. Mean and *SEM* levels of freezing on context test (left panel) and CS test (right panel) in Experiment 3. Rats were subject to a single CS–footshock pairing in a distinctive context. They were then injected with morphine every day for 14 days, commencing either 1 day or 21 days following conditioning. Finally, rats were tested for freezing to the context either 1 day or 21 days after the last injection and freezing to the CS the day after the context test. CS = conditioned stimulus.

injections and test, $F(1, 25) < 1, p > .05$. There was no overall interaction between these two intervals, $F(1, 25) = 2.8, p > .05$. There was no overall difference in levels of freezing observed on context and CS tests, $F(1, 25) < 1, p > .05$. However, there was a significant interaction for the interval between conditioning and the start of morphine injections and levels of freezing on context versus CS tests, $F(1, 25) = 6.4, p < .05$. From inspection it is clear that freezing on context but not on CS test increased as the interval between conditioning and the start of morphine injections increased. There was no interaction between the effect of the interval between last injection and context versus CS test, $F(1, 25) = 3.7, p > .05$. Finally, there was no three-way interaction, $F(1, 25) = 1.4, p > .05$.

Discussion

These results have provided a within-experiment replication of those reported previously. Specifically, they have confirmed that a history of morphine exposures produces a temporally graded retrograde deficit for context fear (Experiment 1) that does not recover when a retention interval is interpolated between the final exposure to morphine and test (Experiment 2). These findings thus reinforce the hypothesis that postconditioning exposures to morphine disrupt the consolidation, rather than the retrieval of context fear conditioning.

Experiment 4

Evidence reviewed at the beginning of this article shows that deficits in context-controlled freezing induced by various postconditioning manipulations (e.g., social isolation or hippocampal lesions) are prevented by preexposures to that context. The previous experiments reported here show that deficits in context-controlled freezing also result from postconditioning exposures to morphine. Therefore, the present experiment studied whether preexposure

likewise protects context fear conditioning from the amnesic effects of postconditioning exposures to morphine. The design consisted in a 2×2 factorial, where the first factor was whether rats were or were not preexposed to the conditioning context and the second factor was whether rats received postconditioning exposures to morphine or saline. Specifically, rats were subjected to daily 2-min preexposures to the conditioning context or to plastic buckets across the 4 days immediately preceding conditioning. They then received context fear conditioning. This consisted in placing the rats in the context and administering an unsignaled footshock 27 s later. The rats remained there for a further 2 min after US delivery. We selected a 27-s placement-footshock interval (rather than the 120-s interval used previously) because work from our laboratories has shown that it is optimal for detecting an influence of context preexposure on context conditioning (e.g., Kiernan & Westbrook, 1993). We administered an unsignaled footshock (rather than the signaled footshock used previously) to show that the retrograde deficits induced by postconditioning exposures to morphine were independent of the use of a signaled footshock. Morphine or saline injections commenced the day after context fear conditioning and lasted for 14 days. Finally, rats were tested for their fear reactions to the context the day after the last injection.

Method

Subjects and apparatus. The subjects were 32 experimentally naive rats (280–350 g) of the same sex and strain and obtained from the same source as Experiment 1. They were kept under the conditions described previously. White plastic buckets (45-cm diameter; 60-cm height) were used. All other apparatuses were those described in Experiment 1.

Procedure. The experiment involved four phases: preexposure, fear conditioning, morphine or saline injections, and testing. Preexposures involved placing rats in the to-be-conditioned context (preexposed groups) or in plastic buckets (not-preexposed groups) for 2 min each day for 4 days. The plastic buckets were located in a room adjacent to the conditioning laboratory. No stimuli were presented during this preexposure. For fear conditioning, all rats were transported to the laboratory, placed in the conditioning context, and shocked 27 s later. The footshock was that used previously. The rats remained in the conditioning context for a further 2 min before being returned to their home cage. On the day following conditioning, and for each of the following 14 days, half of the rats in the preexposed groups and half of the rats in the not-preexposed groups received daily injections of morphine, whereas the remainder received daily injections of saline. These injections were identical to those described previously. Rats were tested for context freezing 1 day following their last injection. This test was 4 min in duration.

Statistics. The data were analyzed by means of three planned, orthogonal contrasts that emerged from the theories being tested. Rats in the not-preexposed-morphine group should display retrograde amnesia and thus differ from rats in the remaining three groups. This retrograde amnesia should be alleviated by context preexposures, and thus rats in the preexposed-morphine group should not differ from rats in the not-preexposed-saline group and rats in the preexposed-saline group. However, rats in the preexposed-saline group should display more freezing (i.e., facilitation as a consequence of precontext exposures) than rats in the not-preexposed-saline group. We did not consider the use of a 2 (preexposed vs. not preexposed) $\times$ 2 (saline vs. morphine) analysis of variance (ANOVA) appropriate because the preexposure manipulation should increase freezing for both morphine- and saline-treated rats and therefore reduce the usefulness of the 2×2 interaction term in evaluating the theories examined.

Results

The mean and *SEM* levels of freezing during the 2-min postshock period on conditioning are shown in the left panel of Figure 4. Inspection of the panel indicates higher levels of postshock freezing among rats preexposed to the conditioning context compared with rats preexposed to the plastic buckets. The mean and *SEM* levels of freezing on test are shown in the right panel of Figure 4. Inspection of the figure indicates that the exposures to morphine across 14 days following conditioning produced retrograde deficits in contextual fear conditioning (not-preexposed-morphine group) that were alleviated by brief context preexposures (preexposed-morphine group).

The statistical analysis confirmed these observations. Analysis of postshock freezing revealed that rats preexposed to the conditioning context displayed significantly more freezing than rats not preexposed to the conditioning context, $t(30) = 4.0$, $p < .01$. Analysis of freezing on test revealed that rats in the not-preexposed-morphine group displayed significantly less freezing than rats in the remaining three groups, $F(1, 28) = 10.5$, $p < .01$. It is important to note that rats in the preexposed-morphine group did not differ significantly in levels of freezing from rats in the not-preexposed-saline and the preexposed-saline groups, $F(1, 28) < 1$, $p > .05$. There was also no significant difference in levels of freezing between rats in these latter two groups, $F(1, 28) < 1$, $p > .05$.

Discussion

This experiment has shown that a series of morphine injections produce retrograde deficits in the fear that accrues from a shocked exposure to a novel context. Thus, the deficit in contextually conditioned fear is independent of whether footshock is signaled, as had been the case in the previous experiments, or unsignaled, as was the case here. It has also shown that these deficits are alleviated by brief exposures to the conditioning context prior to conditioning. This result thus replicates the effects of context preexposure on the retrograde deficits in context-controlled freezing.

Figure 4. Mean and *SEM* levels of postshock freezing (left panel) and freezing on test (right panel) in Experiment 4. Rats were preexposed either to the conditioning context (preexposed group) or to plastic buckets (not-preexposed group) before being subject to a single footshock in the context. Commencing the following day, rats were injected with morphine or saline every day for 14 days. Finally, rats were tested for freezing to the context 1 day after the last injection.

induced by social isolation (Rudy, 1996) or a single injection of morphine (Rudy et al., 1999) in the hours after conditioning as well as the deficits induced by hippocampal damage (Anagnostaras et al., 2001) 1 day or so after conditioning: In all of these cases, preexposure protects context fear conditioning against retrograde impairments. Two additional features of the present data are worthy of comment. First, it might have been expected that the preexposure regime used here could have been ineffective because it occurred within the period identified in Experiment 1 as vulnerable to retrograde amnesia. The present results show that the preexposures were effective. This suggests that the learning resulting from the four context preexposures had been sufficiently consolidated such as to render the context representation invulnerable to the postconditioning morphine exposures. Alternatively, learning about a context may differ from learning about a shocked context with respect to the interval of time required for the consolidation of these representations and, hence, in their vulnerability to postexperiential exposures to morphine. These questions remain to be addressed. The second feature of the data that requires comment was the absence of any evidence for facilitation of contextual freezing on test among saline-treated rats despite evidence for such facilitation in the 2-min postshock period. This outcome was surprising because Kiernan and Westbrook (1993) reported such a facilitation of contextual conditioning both in the immediate postshock period and when rats were tested the day following conditioning. The reasons for this discrepancy are unclear, but one procedural difference between the present experiments and the experiments reported by Kiernan and Westbrook was the interval between conditioning and test. Kiernan and Westbrook tested rats 1 day after conditioning, whereas in the present experiments rats were tested 14 days after conditioning.

Experiment 5

Experiments 1–3 demonstrated that postconditioning exposures to morphine produce a deficit in context-controlled freezing. This deficit was attributed to a disruption by morphine of the processes involved in consolidating an integrated representation of the shocked context. An implication of this hypothesis is that morphine-treated rats should be less able to discriminate between the previously shocked context and a second, different context than saline-treated rats that, presumably, have consolidated an integrated representation of the shocked context. Experiment 4 demonstrated that preexposures protected against the retrograde deficit produced by postconditioning exposures to morphine. This protection suggests that postconditioning exposures to morphine failed to produce a deficit because the integrated representation of the context formed across preexposure had already been consolidated. Therefore, a further implication of this hypothesis concerning the role played by an integrated representation in promoting context discrimination is that preexposures to the conditioning context should not only protect against the deficits in context conditioning among morphine-treated rats but also against the deficits in context discrimination.

The aim of Experiment 5 was to test these predictions. Rats were subjected to daily, 2-min preexposures to the conditioning context or to plastic buckets across the 4 days immediately preceding conditioning. They were then subjected to conditioning. The conditioning procedure was identical to that used in Experiment 4.

Morphine or saline injections commenced the next day and lasted for 14 days. Finally, rats were tested for their fear reactions to both the context where conditioning occurred and to a second, novel context that shared some general features (e.g., shape, presence of a grid floor) with the conditioning context but was otherwise distinct from that context.

Method

Subjects and apparatus. The subjects were 32 experimentally naive rats (280–350 g) of the same sex and strain and obtained from the same source as Experiment 1. They were kept under the conditions described previously. Two sets of four chambers located in different rooms were used. The first set of chambers was that described previously. Each chamber of the second set measured 33 cm (height) $\times$ 31 cm (length) $\times$ 26 cm (width). The side walls and ceiling were made of aluminum, and the back and front walls were made of clear plastic. The side walls and ceiling were painted white, and the outside back wall was covered with white cardboard. The floor consisted of stainless steel rods, 5 mm in diameter, spaced 10 mm apart, center to center. Below the floor was a tray containing bedding material. The chambers were located in separate compartments of a wooden cabinet, whose floor, ceiling, and walls were painted white. The door of each compartment was kept open so as to permit observation of the rat. A concentrated oil (Cara Mia, Sydney, Australia) provided a distinctive rose odor. The oil (1 ml) was sprayed over the bedding material before each session. The room was illuminated by a red fluorescent tube located in the ceiling. All other apparatuses were those used in Experiment 3.

Procedure. The experiment involved four phases: preexposure, fear conditioning, morphine or saline injections, and testing. Preexposures, conditioning, and injections were identical to those described in Experiment 4. The tests for freezing took place on Day 1 (Context Test 1) and Day 2 (Context Test 2) following the last injection. All rats were tested for freezing to both the conditioning context and the second, novel context. Whether rats were tested on Day 1 for freezing to the conditioning context or to the second, novel context was fully counterbalanced across groups. These tests were 4 min in duration.

Statistics. The data were again analyzed by means of planned, orthogonal contrasts. The same between-groups contrasts tested in Experiment 4 were tested here. In addition, a single within-group contrast assessed the differences between levels of freezing in the two contexts. Finally, the interactions between these contrasts were also tested. We did not consider the use of a 2 (preexposed vs. not preexposed) $\times$ 2 (saline vs. morphine) factorial ANOVA appropriate for the same reasons described in Experiment 4.

Results

The mean and *SEM* levels of freezing during the 2-min postshock period on conditioning are shown in the left panel of Figure 5. Inspection of the panel suggests that there were higher levels of postshock freezing among rats preexposed to the conditioning context compared with rats preexposed to the plastic buckets. The mean and *SEM* levels of freezing on tests are shown in the right panel of Figure 5. Inspection of the figure indicates that (a) saline-treated rats discriminated between the conditioning context and the second, novel context because they displayed more freezing in the former; (b) exposures to morphine across 14 days following conditioning produced retrograde deficits in contextual fear conditioning (the not-preexposed–morphine group) that was accompanied by complete generalization of freezing to the second novel context; and (c) brief preexposures to the conditioning context prior to conditioning (the preexposed–morphine group)

Figure 5. Mean and SEM levels of postshock freezing (left panel) and freezing on test (right panel) in Experiment 5. Rats were preexposed either to the conditioning context (preexposed group) or to plastic buckets (not-preexposed group) before being subject to a single footshock in the context. Commencing the following day, rats were injected with morphine or saline every day for 14 days. Finally, the days after the last injection, rats were tested for freezing both to the context where conditioning occurred and in a second, novel context.

alleviated this retrograde deficit and reinstated contextual discrimination.

Analysis of postshock freezing revealed that rats preexposed to the conditioning context displayed significantly more freezing than rats not preexposed to the conditioning context, $t(30) = 4.0$, $p < .01$. This confirms the facilitatory effects of context preexposure observed in Experiment 4.

Analysis of freezing on test revealed that rats in the not-preexposed-morphine group displayed significantly less freezing than rats in the remaining three groups, $F(1, 28) = 5.4$, $p = .03$, averaged across the two context tests. It is important to note that rats in the preexposed-morphine group did not differ significantly in levels of freezing from rats in the not-preexposed-saline and the preexposed-saline groups, $F(1, 28) < 1$, $p > .05$, averaged across the two context tests. This result confirms the results of Experiment 4 and shows that brief preexposures to the conditioning context prior to conditioning alleviates retrograde amnesia. There was also no significant difference in levels of freezing between rats in these latter two groups, $F(1, 28) < 1$, $p > .05$, averaged across the two context tests.

There was evidence for contextual discrimination because there was a significantly greater level of freezing in the conditioning context than in the second, novel context, $F(1, 28) = 32.5$, $p < .01$. However, the not-preexposed-morphine group displayed significantly less contextual discrimination than the remaining three groups because the difference in levels of freezing in the two contexts was significantly smaller for this group than for the remaining three groups. This was shown by the significant interaction between the contrasts assessing differences between (a) the not-preexposed-morphine group versus the remaining three groups and (b) the levels of freezing in the conditioning context and the second, novel context, $F(1, 28) = 7.1$, $p = .01$. If rats in the not-preexposed-morphine group had been able to discriminate between the two contexts, then their levels of freezing when tested in the second, novel context should have been significantly lower than their levels of freezing when tested in the conditioning con-

text. This was not the case. It is important to note that the brief preexposures to the conditioning context reinstated contextual discrimination among rats in the preexposed-morphine group to the levels seen among saline-treated rats. Specifically, there was no interaction between the contrasts assessing differences between (a) the preexposed-morphine group versus the not-preexposed-saline and the preexposed-saline groups and (b) the levels of freezing to the conditioning context versus to the second, novel context, $F(1, 28) < 1$, $p > .05$. Finally, there was no difference in contextual discrimination between the saline-treated rats preexposed versus not preexposed to the conditioning context, as revealed by the absence of an interaction between the contrasts assessing differences between (a) the preexposed-saline group versus the not-preexposed-saline group and (b) the levels of freezing to the conditioning context versus to the second, novel context, $F(1, 28) = 1.4$, $p > .05$.

Discussion

This experiment has confirmed that postconditioning exposures to morphine produce deficits in context-controlled freezing and that these deficits are alleviated by context preexposures. It has also revealed two novel findings. First, the deficits in context-controlled freezing were accompanied by failures of discrimination: Rats subjected to postconditioning morphine exposures froze just as much in the previously shocked context as they did in the second, different context. Second, this failure of discrimination was also reversed by context preexposures: Rats preexposed to the context and subjected to postconditioning morphine exposures not only froze more in the previously shocked context, they also froze less in the second context.

This conclusion regarding a failure of discrimination among rats subjected to morphine exposures after conditioning in a novel context could be challenged on the grounds that floor effects served to obscure any such discrimination. However, this can be rejected for two reasons. First, across the 4 min of test in the second, novel context freezing among rats in the not-preexposed-saline and the not-preexposed-morphine groups did decrease significantly from 30% to 9%, $F(1, 14) = 7.2$, $p = .01$. Thus, the failure of discrimination among rats in the not-preexposed-morphine group was observed independently of significant variations in levels of freezing. Second, previous studies from this laboratory (Kiernan & Westbrook, 1993; Westbrook, Good, & Kiernan, 1997) showed that extensive (4×20 min) context preexposures significantly reduced levels of freezing in the conditioning context (i.e., they produced latent inhibition). The levels of freezing shown by these preexposed animals were similar to the levels of freezing observed here among the not-preexposed-morphine group. Yet even though fear of the conditioning context was very low, rats subject to this latent inhibition manipulation still displayed significantly less freezing in a second, novel context (Kiernan & Westbrook, 1993; Westbrook et al., 1997). In other words, low levels of freezing in the conditioning context do not inevitably reduce the ability to detect significant evidence for contextual discrimination.

A second feature of the present results that requires comment is the failure to provide evidence for facilitation and increased perceptual learning among rats in the preexposed-saline group relative to the performances of rats in the not-preexposed-saline

group. Although there was evidence that the preexposures to the conditioning context facilitated postshock freezing among these rats, there was no evidence for such facilitation when they were tested 2 weeks after conditioning. Moreover, there was also no evidence for an increased discrimination between the conditioning context and the second, novel context among saline-treated rats preexposed to the conditioning context. Both Kiernan and Westbrook (1993) and Westbrook et al. (1997) provided evidence for such facilitation and increased discrimination among preexposed rats under similar conditions to those used here. Again, reason for this discrepancy is unclear but could be related to differences in the interval between conditioning and test (see Experiment 4). In conclusion, the important results from this experiment are that retrograde amnesia for contextual fear conditioning among morphine-treated rats is accompanied by a failure of contextual discrimination and that brief exposures to the conditioning context prior to conditioning prevents both of these effects.

General Discussion

A variety of manipulations imposed on rats in the hours (e.g., social isolation), days, indeed, weeks (e.g., hippocampal damage) following a fear-conditioning episode selectively impair subsequent performance to the context where conditioning took place (for review, see Holland & Bouton, 1999). A common interpretation of these retrograde deficits in contextual conditioning is that they reflect interference with the rehearsal or consolidation of a contextual representation. This rehearsal or consolidation of the contextual representation is critical for a permanent memory of context fear. The experiments reported here tested four predictions that follow from this consolidation-based interpretation of retrograde amnesia. The first prediction was that just as a variety of manipulations administered in the minutes to hours following conditioning impair contextual learning, so too should a variety of events, not just destruction of neural tissue, administered in the days to weeks following conditioning also impair contextual learning. The second prediction was that performance to the conditioning context should not recover across a retention interval. The third prediction was that retrograde amnesia for contextual learning should be accompanied by deficits in contextual discrimination. The final prediction was that contextual learning and discrimination should be protected by brief preexposures to the conditioning context.

The present experiments used a Pavlovian fear conditioning procedure to test these predictions. In Experiments 1–3 rats received a single CS (clicker)–US (footshock) pairing in a distinctive context. In Experiments 4 and 5 rats received an unsignaled footshock in either a novel or relatively familiar context. Rats then received daily injections of morphine or saline commencing at varying intervals (1 to 21 days) after this conditioning episode. Finally, the fear (freezing), elicited by reexposure to the conditioning context and then by presentation of the CS in a different context, was assessed at varying intervals (1 to 21 days) following the last injection. Injection of morphine was chosen as the amnesic agent for two reasons: (a) Morphine mimics the physiological mechanisms of many amnesic manipulations including social isolation (Rudy et al., 1999) and immune activation (McNally et al., 2000), and (b) prolonged exposures to morphine impair phys-

iological mechanisms typically invoked as substrates for learning (e.g., long-term potentiation) and memory (e.g., neurogenesis).

Experiment 1 revealed that a history of morphine injections impaired freezing to the context where conditioning occurred. This deficit was temporally graded such that recent (i.e., 1 day old) but not remote (i.e., 14 days old) contextual memories were impaired. This syndrome was also specific such that morphine injections impaired memory for the context where CS–footshock pairings occurred while sparing memory for the CS–US association. Experiment 2 revealed that memory for the contextual conditioning episode that took place 1 day prior to the start of morphine injections did not recover when an interval was interpolated between the cessation of opiate dependence and test. Experiment 3 confirmed that the interpolation of an interval between the cessation of morphine injections and test did not affect the temporal gradient of retrograde amnesia. Experiment 4 showed that retrograde deficits in conditioning could be alleviated if rats received brief exposures to the to-be-conditioned context. Finally, Experiment 5 showed that the retrograde deficits were accompanied by a failure of contextual discrimination and that both contextual conditioning as well as discrimination could be protected by brief preexposures to the conditioning context.

The chronic exposures to morphine used in these experiments would be expected to have numerous effects on the rats. These include the development of dependence and thus withdrawal when the injections were terminated; alterations in autonomic, endocrine, and cardiovascular function; as well as alterations in locomotor activity. It is worth considering whether the present results could simply be nonspecific consequences of these changes. For example, one class of explanation for deficits in fear conditioning, as indexed by freezing, emphasizes the role played by hyperactivity in disrupting the conditioned response (Gewirtz, McNish, & Davis, 2000; Good & Honey, 1997; McNish, Gewirtz, & Davis, 1997; Richmond et al., 1999). Manipulations that produce hyperactivity, such as hippocampal damage, also impair contextual freezing (e.g., Anagnostaras et al., 1999; Maren & Fanselow, 1997; Teitelbaum & Milner, 1963), and this hyperactivity could compete with freezing for behavioral expression.

However, the present results cannot be explained by any such nonspecific actions for at least three reasons. First, retrograde deficits in freezing were specific to contextual conditioning and were not reliably observed in levels of CS freezing. Second, retrograde deficits in freezing were observed not only as differences between morphine-treated and saline-treated rats but also as differences between rats with identical histories of morphine injections tested at either the same time or at different times relative to both their last injections and the conditioning episode. Finally, rats preexposed to the context developed fear conditioning to the shocked context and discriminated between that and a second context in spite of their postconditioning exposures to morphine. Thus, deficits in context-controlled freezing are not an obligatory consequence of postconditioning exposures to morphine, as would appear to be implied by this performance-based account. Rather, whether such deficits are observed depends on what has been learned about the context prior to its association with footshock.

Exactly what is it that rats learn across preexposure that protects against the amnesic effects of postconditioning exposures to morphine, thereby rendering such rats the equivalent of normal rats shocked in a novel context and treated with saline? Westbrook et

al. (1997) invoked the model of stimulus representation described by McLaren et al. (1989) to explain this learning. The McLaren et al. (1989) model views the experimental context as comprising a set of elements (e.g., kinesthetic, visual, spatial, olfactory) that are represented in terms of patterns of activation across a set of units. At any given moment, the animal samples a subset of the contextual elements, and associations are formed between their conjointly active units using the delta rule (Rescorla & Wagner, 1972). Across repeated exposures to the context, units that are conjointly active will be strongly associated, whereas units activated only occasionally will fail to enter into a stable association. Through this process the animal is held to extract a central tendency of the array of cues comprising the experimental context. The subsequent sampling of any contextual element corresponding to a part of this unitized representation will result in conditioned performance, whereas sampling elements unique to other contexts will fail to elicit significant responding. Westbrook et al. (1997) noted that this model readily explains the positive relation between freezing and the placement-footshock interval as well as the facilitatory effects on both conditioned responding and discrimination of brief preexposures to the conditioning context (Fanselow, 1990; Kieran & Westbrook, 1993). This model thus implicates two potential candidates as mechanisms for impairments on context-controlled freezing and context discrimination. The first is a deficit in the processes controlling sampling among the subset of cues comprising the context, and the second is a deficit in the use of the delta rule to extract the central tendency.

O'Reilly and Rudy (2001) have also described a model of stimulus representation that addresses the question of what rats might learn across preexposure to protect against the amnesic effects of postconditioning exposures to morphine. This model invokes complementary learning—memory systems in the hippocampus and cortex. Whereas the cortex represents stable properties of repeated experiences by learning slowly across a long period of time, the hippocampus represents specific features of individual experiences by rapidly constructing conjunctive representations of these experiences. Although the cortex and hippocampus can use the same conjunctive strategy to represent information, these conjunctive representations are formed automatically in the hippocampus as a consequence of the animal sampling its environment. Thus, the exploration that accompanies placement in a novel context results in the animal sampling the array of individual contextual features. At any given moment, co-occurring features are sparsely represented by a small number of selective hippocampal units that bind these features into a conjunctive representation. Across repeated samplings the cortex represents and permanently stores these stimulus conjunctions. Performance, that is, the use of a stored conjunctive representation, is a function of competition between two processes that depend on both hippocampal and cortical activation. The first is pattern separation. This reduces any interference from related conjunctive representations and therefore underpins discrimination between contexts. The second is pattern completion. This allows retrieval of a stored conjunctive contextual representation from the sampling of a subset of its features and therefore underpins generalization between contexts. This model thus identifies deficits in pattern separation (or enhanced pattern completion) as causal to deficits in context-controlled freezing and context discrimination.

Both the McLaren et al. (1989) and the O'Reilly and Rudy (2001) models provide formal descriptions of the associative processes that might mediate contextual learning. Yet neither model appears to offer an adequate explanation of the present results because rats were subjected to morphine exposures after context fear conditioning. One problem, therefore, is that neither model provides any principled reason as to why contextual fear conditioning and context learning should remain vulnerable to interference up to at least 7 days following conditioning. Just such a reason has been offered by McClelland, McNaughton, and O'Reilly (1995) who suggested that recently acquired memories are stored initially as patterns of synaptic change in the hippocampus. Hippocampal activity, in turn, permits the long-term storage of these memories in the cortex. This long-term storage is identified with the repeated activation of distributed cortical synapses by the hippocampus in the days to weeks to months following the learning episode. According to this model, retrograde amnesia emerged in the present experiments because the repeated exposures to morphine commencing 1–7 days after conditioning inhibited the hippocampal-dependent accumulation of cortical changes representing the conditioning context. Repeated exposures to morphine commencing 7–14 days after conditioning failed to produce retrograde amnesia because the conditioning context was already represented in terms of alterations of cortical synaptic weights.

The exact nature of any such disruption of hippocampal function remains to be determined. The MOR is the primary receptor target for morphine. Binding of morphine to the MOR initiates multiple signal transduction events, but one important pathway relates to cyclic AMP (cAMP) signaling (for review, see McNally & Akil, 2002). Activation of the MOR inhibits adenylyl cyclase and decreases intracellular cAMP. Given the role accorded the cAMP response-element binding protein (CREB) in long-term memory formation (e.g., Bourchouladze et al., 1994), it is possible that chronic exposures to morphine produced retrograde amnesia because they repeatedly inhibited hippocampal cAMP activity. However, other potential mechanisms for this disruption exist. For instance, chronic exposures to morphine increase activation of NMDA receptors (Trujillo & Akil, 1991). In the spinal cord this increased NMDA receptor activation impairs glutamate homeostasis (Mao, Sung, Ji, & Lim, 2002a) and leads to apoptosis (Mao, Sung, Ji, & Lim, 2002b). Similar changes may occur in the hippocampus in response to morphine exposures. Indeed, as described previously, these exposures impair hippocampal long-term potentiation and neurogenesis, putative mechanisms for synaptic plasticity and neuronal growth. Although these mechanisms are speculative, the present results suggest that hippocampal MOR activation may serve as a useful preparation for linking anatomical, cellular, and molecular mechanisms for the consolidation of information in long-term memory.

In conclusion, the present experiments have shown that a history of morphine injections spares fear conditioning to a CS but produces a temporal gradient of retrograde amnesia for contextual conditioned fear. This retrograde deficit was not alleviated by interpolation of a retention interval between injections of morphine and test and was accompanied by a failure of contextual discrimination. Both contextual conditioning and contextual discrimination could be reinstated by brief preexposures to the conditioning context prior to conditioning. These results are consistent with the notion that the context where conditioning occurs is subject to

prolonged processing in memory after conditioning. They are also consistent with the effects of hippocampal damage and with a model (McClelland et al., 1995) that instantiates this postconditioning memorial processing in the hippocampus.

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