Opioid Receptors Regulate Retrieval of Infant Fear Memories:
Effects of Naloxone on Infantile Amnesia

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The authors examined the role of the endogenous opioid system in infantile amnesia for contextual fear conditioning. Rats that were 18 days of age received an aversive footshock in a novel context. Rats displayed conditioned fear when tested 1 min after training but not 24 hr after training. Systemic injection of the opioid receptor antagonist naloxone prior to test, but not immediately after training, alleviated infantile amnesia. Naloxone also alleviated infantile amnesia when injected prior to test 7 days after training. These effects of naloxone were due to actions on central rather than peripheral opioid receptors and were not due to any tendency of the drug to produce fear or freezing. These results show that central opioid receptors regulate retrieval of fear memories in infant rats.

**Keywords:** opioid, fear conditioning, infantile amnesia, memory, freezing
scopolamine (Flood, Cherkin, & Morley, 1987), and amygdaloid stimulation (Liang, Messing, & McGaugh, 1983).

The endogenous opioid system is active from birth (Blass, Jackson, & Smotherman, 1991) and has a critical role in normal infant development (Blass, 1996; Nelson & Panksepp, 1998). Furthermore, behaviors specific to infancy, such as suckling and play, have been found to activate the opioid system. Intraoral infusions of milk in young rats reduce distress vocalizations and decrease sensitivity to noxious stimulation. These effects are prevented by opioid receptor antagonists (Blass & Fitzgerald, 1988). Play behavior may also activate the opioid system because opioid receptor antagonists reduce social play, and conversely, opioid agonists increase it (Beatty & Costello, 1982; Panksepp, Jalowiec, DeEskinazi, & Bishop, 1985; Vanderschuren, Niesink, Spruijt, & Van Ree, 1995; Vanderschuren, Spruijt, Hol, Niesink, & Van Ree, 1996). Finally, there is recent evidence that the endogenous opioid system can play a role in learning and memory in young rats (e.g., Roth & Sullivan, 2001, 2003, 2006; see General Discussion section).

Given the documented role of the endogenous opioids in memory processing, and the effect of common infant behaviors on the opioid system, we studied the role of the endogenous opioids in the development and the expression of infantile amnesia for contextual fear conditioning. In Experiment 1, we replicated the procedures of Rudy and Morledge (1994) to study infantile amnesia for contextual fear conditioning. Rats received a shocked exposure to a context and were tested for fear reaction to the context 1 min or 24 hr later. In Experiment 2, we studied the effect of postconditioning exposures to the opioid receptor antagonist naloxone (0.25 or 2.5 mg/kg) on the development of infantile amnesia for contextual fear. In Experiment 3, we used a 2 × 2 factorial design to study the influence of postconditioning and pretest exposures to naloxone on the development and expression of infantile amnesia for contextual fear. In Experiment 4, we studied whether injection of naloxone would produce fear in untrained rats. In Experiment 5, we studied whether the effects of pretest administrations of naloxone on infantile amnesia for contextual fear could be observed at a longer retention interval (7 days). In Experiment 6, we studied the involvement of opioid receptors in the peripheral versus central nervous systems in opioid regulation of contextual fear memory. Finally, in Experiment 7, we examined whether pretest injections of naloxone enhanced freezing when rats were tested in a context different from that used for training.

Method

Subjects

Experimentally naive, male and female Sprague–Dawley rats obtained from the School of Psychology’s breeding colony at the University of New South Wales were used. Rats were 17–18 (±1) days of age and weighed 35–58 g at the time of training. No more than 1 rat was used from a litter for any one condition. All subjects were housed in litters of 8, with their mother in plastic boxes (30 cm long × 45 cm wide × 16 cm high), in a room with a 12-hr light–dark cycle (lights on at 0600), and food and water were available ad libitum. All rats were treated in accordance with the principles of laboratory animal use published by the American Psychological Association, and all procedures were approved by the Animal Care and Ethics Committee at the University of New South Wales.

Drugs

Naloxone hydrochloride (N7758, Sigma-Aldrich, Sydney, Australia) and naloxone methiodide (N129, Sigma-Aldrich, Sydney, Australia) were dissolved in 0.9% (weight/volume) sterile physiological saline. Naloxone, naloxone methiodide, and saline were injected in a volume of 1 ml/kg.

Apparatus and Procedure

Conditioning for all experiments occurred in a set of two identical Perspex chambers (18 cm long × 15 cm wide × 10 cm high) with a grid floor (5 mm between each 3 mm wide bar), three black and white striped walls, one transparent wall, and a black lid. Each cage was 12 cm above the floor in one of two identical sound- and light-attenuating wooden cabinets. The cabinets were illuminated by a small incandescent light bulb (15 W) located on the rear wall, and a ventilation fan provided a 60-dB ambient noise level in each cabinet. For conditioning, rats were placed into an experimental chamber for 120 s and then received a 1s, 0.6 mA shock. Rats were removed after 30s and returned to their home cage either directly or after receiving a drug injection.

In Experiments 1–6, testing occurred in the conditioning context. In Experiments 7a and 7b, testing occurred in either the conditioning context or an alternative context. The alternative context consisted of a rectangular chamber (12 × 9 × 9 cm) constructed of Plexiglas (FX Plastics, Sydney, Australia) and stainless steel bars. The front wall, rear wall, and ceiling were constructed of clear Plexiglas. The two side walls were made of 3-mm stainless steel rods, spaced 1 cm apart. The rods were vertically positioned relative to the floor of the chamber. The floor of the chamber consisted of a sheet of Plexiglas with 18 holes (6 mm in diameter), spaced 1.5 cm apart, drilled into it. The chamber was elevated 14 cm above the floor of one of the same sound- and light-attenuating wooden cabinets used for the conditioning cages. The cabinets were illuminated by a small, red incandescent light bulb (15 W) located on the door, and a ventilation fan provided a 60-dB ambient noise level. At test, rats were placed back into the experimental chamber for a total of 3 min; no shock was given at test.

Experiment 1

Rats were conditioned and then returned to the home cage for either 1 min (n = 10) or 24 hr (n = 10) before being returned to the conditioning context for test.

Experiment 2

Rats were conditioned and then immediately given a subcutaneous (sc) injection of either saline (n = 8), 0.25 mg/kg naloxone (n = 8), or 2.5 mg/kg naloxone (n = 8) and then returned to the home cage for 24 hr prior to test.

Experiment 3

The design was a 2 (saline vs. naloxone) × 2 (saline vs. naloxone) factorial in which the first factor referred to the type of injection given after conditioning, and the second referred to the type of injection given prior to test. Immediately after conditioning, rats were given an sc injection of either saline or 5 mg/kg naloxone and then returned to the home cage for 24 hr. Then, 10 min prior to test, rats received an sc injection of either saline or 5 mg/kg naloxone.

Experiment 4

Rats were placed in the conditioning context for 150 s but did not receive a shock prior to being returned to their home cage for 24 hr. Rats were injected sc with either saline (n = 6) or 5 mg/kg naloxone (n = 6) 10 min prior to test.
Experiment 5

Rats were conditioned and then returned to the home cage for 7 days. They received an injection of either saline \((n = 10)\) or 5 mg/kg naloxone \((n = 10)\) 10 min prior to test.

Experiment 6

Rats were conditioned and then tested after a 24-hr retention interval. Rats were injected intraperitoneally (ip) with either saline \((n = 11)\), 5 mg/kg naloxone \((n = 11)\), or 5.87 mg/kg (this dose was equimolar to the dose of naloxone) naloxone methiodide \((n = 11)\) 10 min prior to test.

Experiment 7a

Rats were conditioned and then tested after a 1-min retention interval. Testing occurred in either the conditioning context (same, \(n = 6\)) or the alternative context (different, \(n = 6\)).

Experiment 7b

Rats were conditioned and then tested after a 24-hr retention interval. Rats were injected ip with saline or 5 mg/kg naloxone 10 min prior to test. The design was a 2 (saline vs. naloxone) \(\times\) 2 (same context vs. different context) factorial in which the first factor referred to the type of injection given prior to test, and the second referred to the test context. This produced four groups: saline–same, saline–different, naloxone–same, and naloxone–different (all \(ns = 7\)).

Data Analysis

All sessions were video recorded and scored for levels of freezing. Freezing was characterized by an absence of all body movements except those required for breathing and was measured with a time sampling method whereby an observation was made every 3 s, and the rat was scored as freezing or not freezing (Fanselow, 1980). The percentage of observations scored as freezing on test was analyzed by means of analyses of variance (ANOVAs) or independent groups \(t\) tests. A random sample of 20% of the rats tested in this study was scored by an observer who was blind to experimental conditions; the interrater correlation on these rats was .97.

Results and Discussion

Experiment 1: Infantile Amnesia for Contextual Fear Conditioning

The mean (plus or minus the standard error of the mean) percentage of observations scored as freezing from Experiment 1 indicates the presence of infantile amnesia (see Figure 1). Rats 18 days of age tested 1 min after conditioning displayed robust freezing, whereas fear was reduced when rats were tested 24 hr after conditioning. An independent samples \(t\) test confirmed significantly higher levels of freezing when rats were tested 1 min after conditioning than when tested 24 hr after conditioning, \(t(18) = 3.33, p < .01\). These data demonstrate that rats 18 days of age display significant amnesia in a contextually conditioned freezing paradigm when the retention interval is 24 hr. Furthermore, because rats of the same age demonstrate a conditioned freezing response 1 min after training, amnesia in the 24-hr group cannot be due to an acquisition failure. Thus, amnesia exhibited in the 24-hr group was either due to impairment of consolidation or retrieval of the long-term memory of the conditioning episode.
naloxone does not produce a freezing response, as has also been shown in adult rats (McNally & Westbrook, 2003a).

Experiment 5: Effects of Pretest Naloxone at a 7-Day Retention Interval

The mean (plus or minus the standard error of the mean) percentage of observations scored as freezing from Experiment 5 is shown in Figure 5 and indicates that even after a 7-day retention interval, rats that received a pretest injection of naloxone displayed significantly higher levels of conditioned freezing than rats that received saline, \( t(18) = 2.45, p < .05 \). This confirms that the influence of naloxone on infantile amnesia for fear memories can be observed at a longer retention interval.

Experiment 6: Role of Peripheral Versus Central Opioid Receptors in Infantile Amnesia

The mean (plus or minus the standard error of the mean) levels of freezing from Experiment 6 are shown in Figure 6 and indicate that injections of the quaternary opioid receptor antagonist naloxone methiodide did not alter levels of freezing on test (note that one rat from the naloxone methiodide group was excluded from the statistical analysis for having a freezing score that was six standard deviations above the mean). The one-way ANOVA yielded a significant difference among groups on mean levels of freezing at test, \( F(2, 31) = 3.97, p < .05 \). Post hoc analysis, with the Student Newman–Keuls test (\( p < .05 \)), revealed that rats in the saline and naloxone methiodide conditions did not significantly differ from each other on mean level of freezing, but rats in both these groups displayed significantly less conditioned freezing than the naloxone group. This finding suggests that peripheral opioid receptors are not involved in infantile amnesia because selective antagonism of these peripheral receptors did not alter levels of fear on test. By contrast, antagonism of both central and peripheral opioid receptors with naloxone did alleviate infantile amnesia.

Experiment 7: Context Specificity of Effects of Pretest Naloxone

The mean (plus or minus the standard error of the mean) levels of freezing from Experiment 7a are shown in Panel A of Figure 7.
and indicate that those rats tested in the training context exhibited substantially more freezing than those tested in a different context, $t(10) = 3.78$, $p = .004$. The performance of the rats in the “same” condition replicate the 1-min data from Experiment 1, whereas the performance of the rats in the “different” condition shows that the freezing observed in the “same” rats is associatively mediated (i.e., it is not merely a consequence of recently being shocked).

The mean (plus or minus the standard error of the mean) levels of freezing from Experiment 7b are shown in Panel B of Figure 7. A 2 × 2 ANOVA of these data yielded significant main effects for both drug and context, smallest $F(1, 24) = 7.26$, $p = .013$, as well as a significant Drug × Context interaction, $F(1, 24) = 7.26$, $p = .013$. The interaction was due to the rats in the naloxone–same condition exhibiting significantly more freezing than the rats in the other three groups ($p < .05$, Student Newman–Keuls test); performance by rats in the other three groups did not differ. These findings show that the effect of pretest injections of naloxone is not due to a nonspecific enhancement of freezing. That is, those rats given pretest injections of naloxone prior to being tested in the different context exhibited essentially no freezing at test. These results, along with those reported with naïve rats in Experiment 4, strongly suggest that the reported alleviation of infantile amnesia by pretest injections of naloxone is not due to a simple performance effect of the drug.

General Discussion

In these experiments, we studied the role of endogenous opioids in regulating retrieval of infant fear memories. The results can be summarized succinctly. When subjected to context-shock pairings at 18 days of age and tested 1 min after training, rats showed robust fear as indexed by the species-typical defense response of freezing (Experiments 1 and 7a). However, when subjected to context-shock pairings at 18 days of age and tested 24 hr after training, rats showed very little fear. This is additional evidence for infantile amnesia for contextual fear conditioning and replicates the findings of Rudy and Morledge (1994). Amnesia for context fear was mediated, at least in part, by endogenous opioids because it could be alleviated by the opioid receptor antagonist naloxone. Specifically, we found that antagonizing opioid receptors immediately after conditioning did not prevent the development of amnesia for the conditioning episode (Experiment 2), however, antagonizing opioid receptors prior to test facilitated retrieval of the context memory (Experiments 2, 3, and 7b). This effect of pretest injections of naloxone was not due to any tendency of the drug to induce fear or the freezing response because rats exposed to the context without the shock unconditioned stimulus did not freeze at test when they were injected with saline or naloxone (Experiment 4). Furthermore, rats did not freeze following naloxone injections if tested in a context different from where training occurred (Experiment 7b). Instead, the pretest injection of naloxone appears to have facilitated retrieval of a specific context fear memory. Amnesia for contextual fear conditioning could also be alleviated by pretest injections of naloxone when the retention interval was increased to 7 days (Experiment 5). This role for opioid receptors in infantile amnesia appeared to be due to opioid receptors in the central nervous system because pretest injection of naloxone me-

![Figure 6](image_url)

**Figure 6.** Mean percentage of observations scored as freezing during a 24-hr retention test after rats 18 days of age received an injection of saline (Sal), 5.87 mg/kg naloxone methiodide (Meth), or 5 mg/kg naloxone (Nal) just prior to test. Error bars represent standard error of the mean.

![Figure 7](image_url)

**Figure 7.** (A) Mean percentage of observations scored as freezing during a 3-min retention test that occurred either 1 min or 24 hr following a single context–shock pairing in rats 18 days of age. Rats were tested either in the training context (same group) or in an alternative context (different group). (B) Mean percentage of observations scored as freezing during a 24-hr retention test after rats 18 days of age received an injection of saline (Sal) or 5 mg/kg naloxone (Nal) just prior to test. Some rats were tested in the training context, whereas others were tested in a different (Diff) context. Error bars represent standard error of the mean.
The primary finding from these experiments is that opioid receptors regulate retrieval of infant fear memories. This is the first demonstration, to our knowledge, that endogenous opioids regulate retrieval of contextual fear memory in infant rats, and this study further supports the notion that infantile amnesia is due, at least in part, to a retrieval failure. That is, previous studies have shown that infantile amnesia can be alleviated by reinstatement (e.g., Campbell & Jaynes, 1966; Haroutunian & Riccio, 1977) and reminder treatments (e.g., Miller et al., 1991) as well as pretest pharmacological manipulations (e.g., Flint & Riccio, 1997; J. H. Kim et al., 2006). The current data add to this body of evidence indicating that infant rats are able to acquire and consolidate fearful memories, but they cannot readily retrieve these memories after a retention interval.

It is not immediately clear whether the role we have documented for endogenous opioids in the retrieval of learned fear memories is unique to infant rats. There have been relatively few studies examining the role of opioids in memory retrieval in adult rats, and the available evidence is conflicting. For example, studies of conditioned fear in adult rats have reported that systemic or intracerebral administration of opioid receptor antagonists does not alleviate retrieval deficits for fear memories when those deficits are produced by interference from new learning (McNally, Pigg, & Weidemann, 2004; McNally & Westbrook, 2003a). In contrast, pretest administration of opioid receptor antagonists can enhance memory for conditioned avoidance in mice (Flood et al., 1987), even after spontaneous forgetting (Ilyutchenok & Dubrovina, 1995). Further research is needed to clarify this issue.

However, Roth and Sullivan (2003) have suggested that the endogenous opioid system has a unique role memory retrieval during early development. Specifically, these investigators have shown that neonatal rats (less than postnatal [PN] Day 9) exhibit a preference for an odor paired with stroking (Roth & Sullivan, 2006) or a mild footshock (Roth & Sullivan, 2003). Slightly older rats (i.e., greater than PN 10) exhibit an aversion to an odor paired with the mild footshock. Of critical importance here, pretest injections of the opioid antagonist naltrexone impaired retrieval of the learned preference in rats younger than 9 days of age whether the odor had been paired with stroking (Roth & Sullivan, 2006) or the mild footshock (Roth & Sullivan, 2003). This finding of impaired retrieval following pretest injection of an opioid antagonist is in marked contrast to the current findings in which pretest naltrexone enhanced retrieval of a contextual fear memory. Furthermore, Roth and Sullivan (2003) also reported that pretest injections of naltrexone did not affect memory retrieval (of an odor aversion) in PN 11–12 rats. In other words, they found that the endogenous opioid system appears to have a role in learning and memory but only during a “sensitive” period occurring before PN 10. Again, this finding is in contrast to the results reported in the current study in which rats were 19 days of age at test (or 25 days of age in Experiment 5). Nonetheless, pretest injections of the opioid antagonist naltrexone reliably enhanced retrieval of the contextual fear memory. Clearly, the endogenous opioid system is involved in learning and memory. Its role may differ depending on the rat’s age and the type of training procedure used. Much more research is needed in this area to clearly sort out these issues.

On the basis of the current study’s results, opioid receptors in the central nervous system appear especially important for regulating infant memory retrieval. Infantile amnesia was alleviated by naltrexone, which antagonizes peripheral and central opioid receptors, but not by naloxone methiodide, which antagonizes peripheral opioid receptors only. There are two arguments against the possibility that our failure to detect an effect of naloxone methiodide on infantile amnesia was due to a failure to test with an effective dose. First, we used a dose of naloxone methiodide that was equimolar to the dose of naloxone. Second, we have previously shown that this dose of naloxone methiodide is effective in modulating pain sensitivity (McNally, Johnston, & Westbrook, 2000). The failure of the peripheral opioid receptor antagonist to alleviate infantile amnesia is interesting because in adult rats, peripheral opioid receptors modulate memory processing. Rudy et al. (1999) showed that activity at peripheral opioid receptors can impair memory consolidation in adult rats. The current data show that central, not peripheral, opioid receptors regulate infant memory retrieval. The precise neuroanatomical locus for this contribution is unclear. One structure that may be critical for the effects of naloxone on infantile amnesia is the hippocampus. The hippocampus is crucial for the formation and retrieval of contextual memories (for a review, see Anagnostaras, Gale, & Fanselow, 2001; O’Reilly & Rudy, 2001; Rudy & O’Reilly, 2001). Opioid peptides and their receptors within the hippocampus play an important role in modulating excitatory neurotransmission and synaptic plasticity (for a review, see Simmons & Chavkin, 1996). Thus, opioid activity within the hippocampus on test may be causal to infantile amnesia for contextual fear. We are currently examining this possibility.

Our data also show that postconditioning injections of an opioid receptor antagonist do not enhance the consolidation of contextual fear memories in infant rats. This result stands in marked contrast to the effects of postconditioning opioid receptor antagonist in adult rats. Postconditioning injections of an opioid receptor antagonist facilitate (Castellano, Introini-Collison, Pavone, & McGaugh, 1989; Castellano & McGaugh, 1989; Flood et al., 1987; Gallagher, 1982; Izquierdo & Netto, 1985; Messing et al., 1979; Netto & Maltchik, 1990; Ukai et al., 1993), whereas postconditioning injections of opioid receptor agonists impair (McNally & Westbrook, 2003b; Rudy et al., 1999; Ukai et al., 1993), consolidation of memories in adult rats. This result also stands in contrast to Roth and Sullivan’s (2001, 2003, 2006) finding that immediate postconditioning injections of naltrexone affect acquisition of odor preferences in rats younger than PN 9. However, they also reported that injections of naltrexone immediately after training did not affect olfactory learning in rats older than PN 10 (Roth & Sullivan, 2001, 2003). In any case, it is unlikely that our failure to detect an effect of postconditioning naloxone on contextual fear memory in rats 18 days of age was due to a failure to test with an adequate dose of the antagonist. Postconditioning injections of 0.25, 2.5, and 5 mg/kg had no effect, whereas pretest injections of 5.0 mg/kg naloxone were effective. It is possible that the duration of opioid receptor antagonism in the postconditioning period was insufficient to facilitate memory consolidation in rats 18 days of age. The half life of naloxone in the rat is approximately 30 min (Ngai, Berkowitz, Yang, Hempstead, & Spector, 1976). Therefore, it is possible that facilitation of memory consolidation might have been observed if opioid receptors had been antagonized for longer in the
current study. However, it is also possible that the role of opioid receptors in memory processing is qualitatively different in young rats than in adult rats. Further research is needed to determine whether the duration of opioid receptor antagonism in the post-conditioning period is a critical variable for opioid receptor modulation of memory storage in rats 18 days of age.

Finally, it is worth noting that the precise mechanisms for alleviation of infantile amnesia by opioid receptor antagonism are unclear. Context fear conditioning involves at least four processes. The first process records the spatial–temporal conjunctions among the several cues composing the context and combines these into a unitary or integrated representation (Fanselau, 1990; McLaren, Kaye, & Mackintosh, 1989; O’Reilly & Rudy, 2001). The second process forms excitatory associations between the output of this configurual system and the fear motivational system (Maren & Quirk, 2004; Schafe, Nader, Blair, & LeDoux, 2001). 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. The third requirement for such control is that the unitary representation of the shocked context be rehearsed, consolidated, or otherwise rendered relatively permanent (Anagnostaras, Maren, & Fanselau, 1999; J. J. Kim & Fanselau, 1992; Maren, Aharonov, & Fanselau, 1997; Pugh et al., 1999, 2000; Rudy, 1996). Finally, the unitary or integrated contextual representation and fear association must be retrieved on test (O’Reilly & Rudy, 2001; Rudy & O’Reilly, 2001). The formation, storage, and retrieval of an integrated or unitary contextual representation depend heavily on the hippocampus (O’Reilly & Rudy, 2001; Rudy & O’Reilly, 2001). The failure of postconditioning naloxone to prevent infantile amnesia suggests that opioid receptors do not influence the consolidation of contextual fear memories in infant rats. However, it is unclear from the current data whether the pretest administrations of the opioid receptor antagonist alleviated amnesia directly by facilitating retrieval of the context-shock association or whether they alleviated amnesia indirectly by facilitating retrieval or use of the contextual representation.

In conclusion, these experiments show infantile amnesia for contextual fear conditioning when rats 18 days of age are tested for fear memories 24 hr after training. These experiments show for the first time that opioid receptors regulate retrieval of a contextual fear memory in infant rats because expression of infantile amnesia was alleviated by pretest administrations of the opioid receptor antagonist naloxone. The development of infantile amnesia was largely unaffected by postconditioning administrations of naloxone across a wide-dose range.

References


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