

Deletion of the *mu* opioid receptor results in impaired acquisition of Pavlovian context fear

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Abstract

The *mu* opioid receptor may constitute a critical component of a negative feedback system that regulates Pavlovian fear conditioning. We investigated context fear conditioning acquisition and expression in *mu* opioid receptor knockout mice (on an inbred, C57 genetic background). We discovered that the *mu* receptor knockout results in an unexpected and significant deficit in context fear acquisition. Mice lacking the *mu* receptor showed normal fear acquisition when subjected to a 1-day fear conditioning protocol but evinced deficient fear learning when acquisition was conducted across 5 days. The knockout mice showed normal reactivity to footshock in both fear conditioning protocols. Finally, we confirmed the effectiveness of the receptor deletion in the C57 strain by subjecting the mice to a test of morphine analgesia in the hot-plate assay. As has been seen with mixed genetic background, the receptor deletion resulted in a complete lack of analgesic response to 10 mg/kg morphine. Surprisingly, mice with a single copy of the *mu* receptor gene (heterozygous knockouts) showed intact sensitivity to morphine but a significant deficit in Pavlovian fear conditioning. The results indicate that deletion of the *mu* receptor gene impairs fear conditioning and that the conditioning and analgesia effects of heterozygous deletion are dissociable. The conditioning deficit seen in this line of mice may be related to impairment in hippocampus function.

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1. Introduction

Opioid receptors play a crucial role in the regulation of Pavlovian fear conditioning. In rats, peripheral or central application of opioid receptor antagonists disrupts the conditional analgesia that results from Pavlovian fear conditioning (Fanselow, Calcagnetti, & Helmstetter, 1989; Helmstetter & Fanselow, 1987). In Pavlovian fear conditioning, conditional analgesia develops across repeated footshock exposures and results in a negative feedback regulation of fear acquisition (see Fanselow, 1998). Opioid receptors mediate this feedback regulation of Pavlovian

fear conditioning. Application of naloxone, either systemically, centrally, or specifically in the periaqueductal gray, causes enhancement in the development of Pavlovian context fear (Fanselow, 1981; Fanselow et al., 1991; Hammer & Kapp, 1986; Sanders & Fanselow, 2002) by antagonizing the conditional analgesia that develops across fear conditioning trials. In rats, opioid antagonism also attenuates the well-known behavioral phenomenon called blocking, which most likely is mediated by negative feedback regulation of subjective shock intensity (Fanselow, 1998; Fanselow & Bolles, 1979, but see McNally, Pigg, & Weidemann, 2004). Opioid receptors thus most likely represent a major component of the negative feedback regulation of Pavlovian fear conditioning and thus may also represent a molecular substrate for basic rules of learning and memory.

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Opioid receptor knockout (KO) mice have been used extensively to define the role of opioid processes in nociception and analgesia (for an extensive review, see Kieffer & Gaveriaux-Ruff, 2002). *Mu* opioid receptor (MOR) KO mice show enhanced baseline sensitivity to painful stimuli in some tests, such as the tailflick assay (Sora et al., 1997) and paw pressure test (Martin, Matifas, Maldonado, & Kieffer, 2003). Numerous studies have shown the MOR to be crucial for morphine-induced analgesia; MOR KO mice show a complete absence of analgesia after morphine administration (Matthes et al., 1996; Sora et al., 1997). The only test of stress-induced analgesia thus far in MOR KO mice reported a selective deficit in a late phase of analgesia produced by swim stress (LaBuda, Sora, Uhl, & Fuchs, 2000). While not completely analogous to fear conditional analgesia, these results suggest the possibility that the feedback regulation of Pavlovian fear conditioning might be compromised in MOR KO mice. Few studies have investigated learning and memory processes in MOR KO mice. To date, two laboratories have investigated MOR KO mice in the Morris Water Maze, a standard laboratory test of learning and memory. MOR disruption seems to impair acquisition (Jamot, Matthes, Simonin, Kieffer, & Roder, 2003) as well as long-term memory for spatial information (Jang et al., 2003) in this task. An initial report on MOR KO mice reported that they show less freezing than their wild-type controls in a stress-induced depression procedure involving exposure to 36 footshocks (Filliol et al., 2000). Those authors did not investigate the acquisition of fear conditioning per se and used many more shocks than is typical in a conditional fear procedure. Thus far, no one has investigated the acquisition of fear conditioning in these animals using standard Pavlovian fear conditioning parameters. That is, no one has tested them in fear conditioning procedures where opioid receptors are known to play a crucial role in acquisition. Additionally, very little work has been done on this genetic manipulation on an inbred background. Standardization of genetic background is essential to discerning the behavioral effects of genetic manipulations (Banbury Conference on Genetic Background in Mice, 1997). Therefore, we used a line of MOR KO mice that has been backcrossed to the C57 strain for at least 10 generations. The C57 strain has been used extensively in fear conditioning to evaluate the effects of genetic manipulations (Costa et al., 2002), protein kinase manipulations (Ahi, Radulovic, & Spiess, 2004), training parameter manipulations, and sex differences (Wiltgen, Behne, Sanders, & Fanselow, 2001).

In the present set of studies, MOR KO mice were tested for Pavlovian context fear with two different, widely used procedures. In the first study, animals were subjected to a series of five mild footshocks in a single session and tested for context fear 24 h later. This design allowed for direct comparison to recent studies in which

we applied opioid receptor antagonists during acquisition (Sanders & Fanselow, 2002). In the second study, animals were subjected to a single shock each day across 5 days. By generating a more gradual learning curve, this experiment allowed for greater resolution in the analysis of context fear acquisition in these animals. We also measured footshock reactivity in all fear conditioning experiments to assess possible baseline differences in pain sensitivity caused by the receptor deletion. We conducted a hot-plate test of morphine analgesia on these same animals to confirm the effectiveness of the MOR KO. A final study was conducted, with commercially available C57 mice, to validate our measure of footshock reactivity.

2. Experiment 1

As a first assessment of the effectiveness of the receptor deletion, we used a method that we have employed previously to reveal the effects of MOR receptor blockade in C57 mice. We used a simple, single day, conditioning procedure to establish Pavlovian context fear and we tested the animals 24 h after acquisition. We previously have used this methodology to confirm that particular opioid receptor subtypes (including both the MOR and *delta* opioid receptor) regulate Pavlovian fear conditioning in C57 mice (Sanders & Fanselow, 2002).

2.1. Materials and methods

2.1.1. Subjects

Male mice served as subjects in all experiments. Experiment 1 employed animals with a deletion of the *mu* opioid receptor (MOR). The initial production of the line has been described previously (Matthes et al., 1996). The original line of animals was backcrossed to the inbred C57Bl/6 strain for at least 10 generations. All experimental animals were littermates produced by crosses of heterozygous animals. Experimental animals thus included those of wild-type (+/+; $n=6$), heterozygous KO (+/-; $n=12$), and homozygous KO (-/-; $n=5$) genotypes. All mice were bred in the Franz Hall vivarium at UCLA and housed in groups of 2–4 (mixed genotype), in individually ventilated and watered cages with food and water available freely. All procedures were conducted in accordance with the UCLA Chancellor's Animal Research Committee, the US Public Health Service "Policy on Humane Care and Use of Laboratory Animals," and the National Institutes of Health "Guide for the Care and Use of Laboratory Animals."

2.1.2. Apparatus

Animals were run in four identical conditioning chambers (30 cm × 24 cm × 21 cm; Med Associates, St. Albans, VT), used for both training and testing.

The chambers were situated on a stainless-steel rack in a brightly lit room. The ceiling and back wall of each chamber were made of opaque white plastic. The side-walls were constructed of aluminum. The front door of each chamber was made of clear polycarbonate plastic. Each chamber contained a removable grid floor and waste pan. Before the introduction of each squad of mice, each box was cleaned with a 1% acetic acid solution and dried thoroughly. A thin film of the solution was placed in the waste pan as well. The grid floor contained 36 stainless-steel rods (3 mm diameter) spaced 8 mm center-to-center. Each grid floor, when placed in the chamber, made contact with a circuit board through which scrambled shock was delivered. Shock was programmed and delivered through a modular Med Associates system (Med Associates, St. Albans, VT). During all experimental procedures, a common HEPA air filter supplied background noise (55 dB, A scale).

A single camera recorded behavior from animals in all four boxes. Freezing was measured with an automated system whereby continuous video data were analyzed with the aid of NIH Image. This system has been described in detail (Anagnostaras, Josselyn, Frankland, & Silva, 2000), but basically consists in measuring the variance in pixel intensity across successive video frames (taken at 1 Hz) and then computing its standard deviation. A threshold is then applied to the data to yield a percent freezing score. In pilot experiments (data not shown), the threshold for freezing was adjusted to yield 98% agreement with a pair of human observers. All video was recorded to VHS tape. For measurement of the unconditional response to footshock, the video was digitized and stored on a Macintosh G4 at a rate of 10 frames per second. NIH Image was then used to calculate a distance traveled by each subject during each shock (Anagnostaras et al., 2000).

2.1.3. Procedure

Animals were transferred to a holding room (adjacent to the conditioning room) and tailmarked with colored Sharpie pens. After at least 15 min of acclimation to the holding room, animals were transferred to the conditioning room and placed in the conditioning chambers. Animals were allowed 3 min of exploration in the conditioning chamber before the delivery of footshock. Animals received five, 1-s footshocks at an intensity of 0.5 mA and an intershock interval of 1 min. The animals were returned to the conditioning chamber 24 h after training for a test of context fear. During the context test, the behavior of the animals was observed over a 5 min period. Using the aforementioned video analysis system, freezing was measured every 1 s for the 5 min period. Post-shock freezing was measured during the training session, for each of the 1 min periods following the five footshocks. The activ-

ity burst produced by each shock was scored from VHS tape. Each of these three dependent measures was subjected to a one-way between-subjects ANOVA with three levels (genotype: homozygous KO, heterozygous KO, and wild-type).

2.2. Results

Animals of the three genotypes were subjected to Pavlovian context fear conditioning with a series of five footshocks in a single session. Twenty-four hours later, animals were returned to the conditioning chamber for measurement of context freezing. Genotypes did not differ in their unconditional response to footshock [$F(2,20) = 1.03$, $p = .37$] (as depicted in Fig. 1). Genotypes did not differ in their level of freezing during the post-shock periods of the conditioning session [$F(2,20) < 1$] (as seen in Fig. 2). Finally, genotypes did not differ in freezing levels during the 24 h context test [$F(2,20) < 1$] (as depicted in Fig. 3). Thus, MOR deletion has no significant effect on Pavlovian fear produced under the parameters used here.

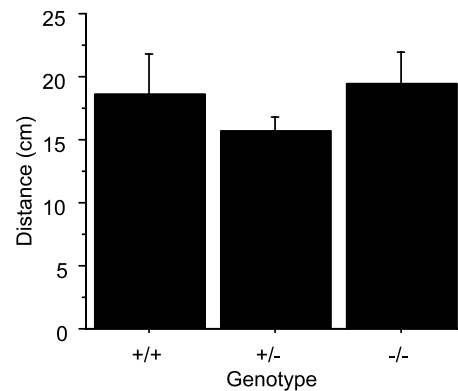


Fig. 1. Mean (+SEM) activity burst in each of the three genotypes, averaged across the five footshocks during acquisition. Values represent the mean distance traveled during the 1-s period of footshock.

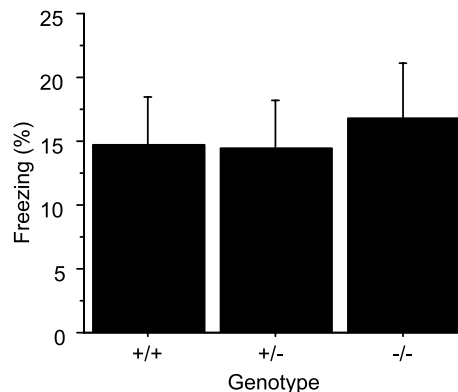


Fig. 2. Mean (+SEM) post-shock freezing in each of the three genotypes, averaged across the five 1-min post-shock periods during acquisition. Values represent the mean percentage of video samples (taken at 1 Hz) scored as freezing.

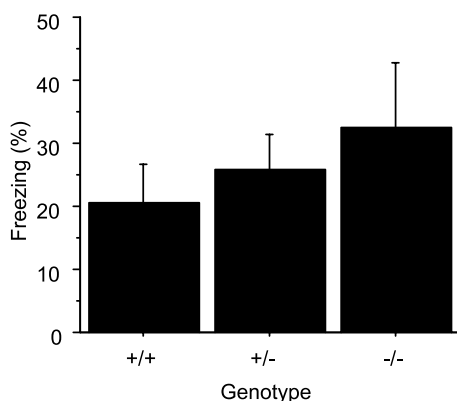


Fig. 3. Mean (+SEM) context freezing in each of the three genotypes, averaged across the five 1-min periods during the 24 h context test. Values represent the mean percentage of video samples (taken at 1 Hz) scored as freezing.

3. Experiment 2

In Experiment 2, we turned to a more sensitive conditioning procedure that produced a more gradual learning curve. This procedure may prove more sensitive in revealing subtle changes in learning produced by the MOR manipulation. Our laboratory previously has used this methodology to examine opioid regulation of acquisition and to examine subtle effects of hippocampus-specific receptor modifications (e.g., Young & Fanselow, 1992). In addition, we subjected the same sample of mice to a hot-plate test of morphine analgesia.

3.1. Materials and methods

3.1.1. Subjects

Experiment 2 employed animals from the same line that was used in Experiment 1. Again, all animals were littermates and the product of heterozygous crosses and included +/+ ($n=10$), +/- ($n=20$), and -/- ($n=17$) genotypes.

3.1.2. Apparatus

The fear conditioning apparatus for Experiment 2 was identical to that of Experiment 1. The hot-plate apparatus consisted of a metal plate kept at 52°C. Animals were placed on the plate within the confines of a Plexiglas cylinder (15 cm inner diameter).

3.1.3. Procedure

In Experiment 2, animals received a single footshock each day for 5 days. Animals were transferred to the holding room each morning, tailmarked, and left undisturbed for at least 15 min. Animals were then transferred to the conditioning room and placed in the conditioning chambers. After a 3 min pre-shock period, a single 1-s footshock (0.6 mA) was given. Freezing was monitored

each day during the pre-shock and post-shock periods. The activity burst produced by each shock was analyzed as well. Freezing during the pre-shock period was taken as the primary measure of context fear acquisition. The freezing data from this period were subjected to a two-way mixed ANOVA, with genotype as a between-groups factor and day as a repeated measure. Both post-shock freezing and activity burst measures were subjected to a one-way ANOVA with genotype as the independent factor.

At least 1 month after the completion of the fear conditioning experiment, the same animals were subjected to the hot-plate test. Half of each genotype received either morphine (10 mg/kg in a volume of 10 ml/kg phosphate-buffered saline) or vehicle injected intraperitoneally. Hot-plate latencies were monitored every 30 min for the next 3 h. Latencies from the 60 min measure (where +/+ animals showed maximal morphine analgesia) were subjected to a two-way ANOVA with genotype and drug treatment as the independent factors.

3.2. Results

To examine the development of conditioning, we subjected mice to a multi-day Pavlovian context conditioning procedure, where freezing was measured during a 3-min period prior to shock for each of 5 days. Each day, mice were placed in the conditioning chambers and given a single shock after a 3-min period. Freezing was measured across days as an indicator of context fear conditioning. As in Experiment 1, genotypes did not differ in unconditional response to any of the five footshocks. Groups exhibited similar mean activity bursts [$F(2,43) < 1$] (as depicted in Fig. 4). Groups also failed to differ in freezing during any of the 1-min post-shock periods and exhibited similar mean post-shock freezing levels [$F(2,44) < 1$] (Fig. 5). However, groups did show

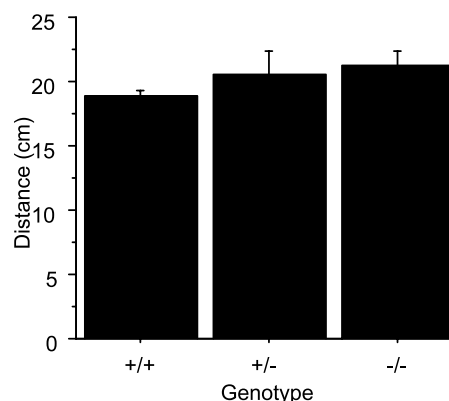


Fig. 4. Mean (+SEM) activity burst in each of the three genotypes, averaged across the five footshocks (one footshock per day across 5 days). Values represent the mean distance traveled during the 1-s period of footshock.

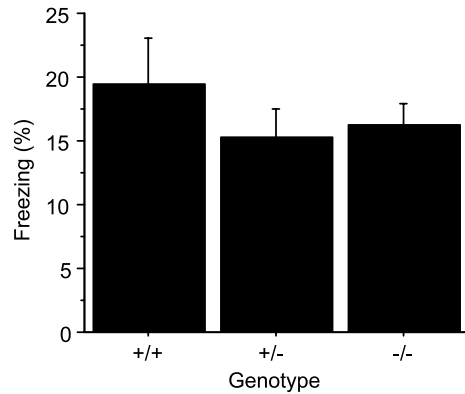


Fig. 5. Mean (+SEM) post-shock freezing in each of the three genotypes, averaged across five 1-min post-shock periods (one post-shock period per day across 5 days). Values represent the mean percentage of video samples (taken at 1 Hz) scored as freezing.

different patterns of freezing during the 3-min pre-shock period across days. A mixed ANOVA revealed a significant effect of genotype [$F(2,44) = 4.82, p < .05$], a significant effect of day [$F(4,176) = 90.06, p < .0001$], and a significant interaction between the two factors [$F(8,176) = 3.29, p < .01$] (as depicted in Fig. 6). Thus, differences among the genotypes emerged across training days and were pronounced only for days 4 and 5. These differences were confirmed with post hoc analyses. Fisher's PLSD revealed a significant deficit in both +/- and -/- animals on day 4 but a significant deficit only in +/- animals on day 5 ($p < .05$).

The same animals later were subjected to hot-plate testing. The animals from each genotype were assigned further into equal groups that received either morphine or vehicle. For 3 h following the injection, latencies were recorded every 30 min for paw licking/jumping. Analgesia was pronounced in both +/+ and +/- animals but absent in -/- animals. For clarity, Fig. 7 presents the data from the 60-min time point, where +/+ animals showed their greatest level of morphine-induced analge-

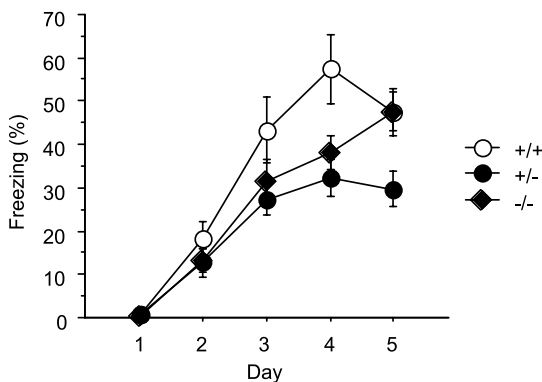


Fig. 6. Mean (\pm SEM) pre-shock freezing in each of the three genotypes for each day of acquisition (one pre-shock period per day across 5 days). Values represent the mean percentage of video samples (taken at 1 Hz) scored as freezing during the 3 min of context exposure before each shock.

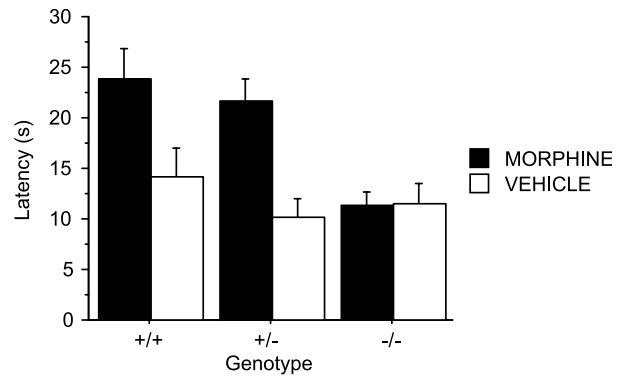


Fig. 7. Mean (+SEM) latency to hind paw lick or jump on the hot-plate test.

sia. At this point, significant differences were found for genotype [$F(2,41) = 5.26, p < .01$], drug [$F(1,41) = 14.49, p < .001$], and the interaction between the factors [$F(2,41) = 4.55, p < .05$]. Morphine analgesia, unlike fear conditioning, was affected only by the complete deletion of the MOR.

4. Experiment 3

Throughout the aforementioned fear conditioning experiments, we failed to see any effect of genotype on the unconditional response to footshock. In Experiment 3, we sought to validate the utility of the activity burst measure for examining the unconditional response to footshock. To this end, we examined the magnitude of activity burst at varying footshock intensities and compared bursts associated with more traditional behavioral measures of footshock sensitivity (flinching, jumping, and vocalizing).

4.1. Materials and methods

4.1.1. Subjects

Experiment 3 employed 20 C57Bl/6NTac mice as subjects. The animals were obtained from Taconic Farms (Germantown, NY) and housed, and treated identically to those in Experiments 1 and 2. The animals were given at least 2 weeks of acclimation to the Franz Hall vivarium before the initiation of the study.

4.1.2. Apparatus

The conditioning apparatus for Experiment 3 was identical to that of the other experiments. Minor additions were made for the on-line measurement of vocalization. First, a microphone contained in the video camera was connected to a monitoring speaker in the adjacent observation room. Second, the microphone was connected also to an oscilloscope, allowing for visual confirmation of vocalizations.

4.1.3. Procedure

Single animals were brought from the Franz Hall vivarium directly into the conditioning room and placed in the conditioning chamber. After a pre-shock period of 1 min, animals received three ascending series of footshocks with an intershock interval of 20 s. Footshock intensity began at 0.04 mA and was increased by 0.04 mA for each shock in the series. Each series ended when the mouse vocalized. The thresholds for flinching, jumping, and vocalizing were recorded. All behavior was recorded on VHS tape for later analysis whereby the activity burst was measured for each animal at that animal's threshold for each behavior. The video was digitized and stored on a Macintosh G4 at a rate of 10 frames per second. NIH Image was then used to calculate a distance traveled by each subject during the appropriate shock (flinching, jumping, or vocalizing) (Anagnostaras et al., 2000). The distance traveled during each shock was then used to determine if the traditional behavioral measures of footshock sensitivity were associated with significantly different activity bursts. The data were subjected to a repeated-measures ANOVA, with the burst at each behavioral threshold as the repeated measure.

4.2. Results

C57Bl/6 animals were subjected to three ascending series of footshocks, terminating at the vocalization threshold. Within each series, and for each animal, a single shock intensity was determined to be the threshold intensity for flinching, jumping, or vocalizing. The activity bursts were compared across the threshold shocks for each behavior. As depicted in Fig. 8, the three behaviors were associated with significantly different activity bursts [$F(2, 38) = 36.99$, $p < .0001$]. Thus, the activity burst measure employed in Experiments 1 and 2 is extremely sensitive to changes in foot-

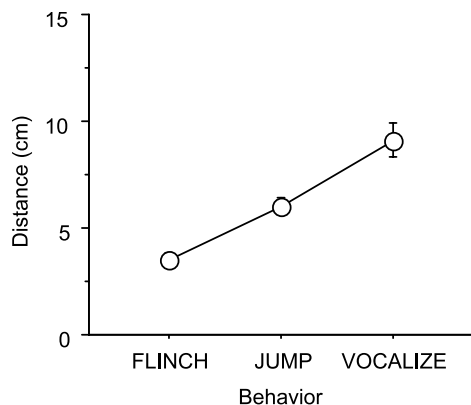


Fig. 8. Mean (\pm SEM) activity burst, in commercially available C57Bl/6 mice, as a function of shock intensity. The three intensities of shock were associated with the threshold for elicitation of one of three traditional measures of footshock reactivity. Values represent the mean distance traveled during the 1-s period of footshock.

shock perception as expressed in the three traditional measures of reactivity.

5. Summary and discussion

The major findings of the current studies are: (1) MOR deletion causes a small but significant impairment in fear conditioning when conditioning is conducted across 5 days, (2) homozygous MOR deletion is sufficient both in impairing fear conditioning acquisition and in eliminating morphine analgesia, (3) heterozygous MOR deletion is sufficient in causing a fear conditioning deficit even though it is insufficient in preventing morphine analgesia, (4) MOR deletion has no significant effect on context fear when conditioning is produced with a single-day protocol, and (5) MOR deletion has no significant effect on footshock reactivity. Regarding footshock activity, the measure we employed admittedly reflects a very basic level of processing by the central nervous system. We can draw no conclusions about the effect of footshock on higher brain structures that might be involved in fear conditioning (Fanselow, 1991). We can only conclude that the MOR deletion affects the conditional fear response without affecting the unconditional response. Previous research had revealed a deficit in freezing in the MOR KO mice when re-exposed to a context in which they received footshock (Filliol et al., 2000). This previous study employed 36 footshocks, many more than we typically use for fear conditioning studies. Our single-day protocol, with a series of five footshocks, failed to confirm this previous finding. However, a 5-day protocol did reveal a deficit in fear conditioning, consistent with the immobility deficit. In the homozygous KO, the deficit appears in the rate of acquisition of conditioning, rather than in the asymptotic magnitude of conditioning. In contrast, the deficit in the heterozygous KO appears in both rate and in magnitude. Thus, the effect of the heterozygous KO appears greater than that of the homozygous KO. In conclusion, the 5-day protocol used here reveals a subtle but significant deficit in context fear acquisition. The deficit revealed in the 5-day "acquisition curve" leads to a characterization of the deficit as a deficit in acquisition of the fear conditional freezing response. However, the behavioral deficit may stem from a defect in consolidation following each training session. Indeed, the post-shock freezing levels are unaffected by the receptor deletion. Thus, one might be tempted to characterize the deficit as a deficit in the acquisition curve (by definition) without evidence of a deficit immediately following the shock on each training day. We do not discount the possibility that consolidation processes might be compromised in the MOR KO mice but we do not have direct evidence of such a deficit. We can conclude only that, over 5 days of training, the animals do show a deficit in the acquisition of the freezing response.

Mu receptors are distributed throughout brain structures involved in sensory, nociceptive, and emotional processing (Moskowitz & Goodman, 1984). The distribution of *mu* receptor binding in neocortical layers I, IV, and VI suggests the involvement of the receptor in sensory input and processing (Moskowitz & Goodman, 1984). The MOR deletion produced by genetic knockout is global and has been verified in every locus where MOR are found. These areas include neocortex, caudate/putamen, nucleus accumbens, amygdala, thalamus, hypothalamus, the periaqueductal gray, and the dentate gyrus of the hippocampus (Kitchen, Slowe, Matthes, & Kieffer, 1997). The distribution of the MOR gene closely matches the receptor distribution and includes the pyramidal cells of the hippocampus, the granule cells of the dentate gyrus, and the amygdala (Kaufman et al., 1995). Clearly, the deficit discovered in the current study might reflect altered function in any number of these structures, which have been implicated in contextual fear learning. The intact footshock reactivity and intact conditioning under particular circumstances argues against a gross deficit in sensory or emotional processes. Although we tested only context conditioning and have no data on tone conditioning in these animals, we propose that MOR disruption in the hippocampus likely underlies the conditioning deficit. Supporting clues about the anatomical location of the present effects can be gleaned from the recent literature on various MOR KO lines of mice. Studies have indicated dysfunctional hippocampal physiology and deficits in spatial learning and memory in these animals. Hippocampal long-term potentiation, a widely studied putative cellular analog of learning, is hypothesized to underlie spatial learning and context conditioning. MOR KO mice are deficient in long-term potentiation (LTP) at both the perforant path-dentate gyrus synapse and the mossy fiber-CA3 synapse. In dentate gyrus, tetanic stimulation produces only a short-lasting potentiation in the MOR KO but a long-lasting potentiation in wild-type controls (Matthies et al., 2000). At the mossy fiber-CA3 synapse, MOR KO results in a lower magnitude LTP that decays faster than in the wild-type controls (Jamot et al., 2003). In contrast, synaptic plasticity appears to be intact at the Schaffer collateral-CA1 synapse of MOR KO mice. Along with these deficits in hippocampal physiology, MOR KO mice also display deficits in spatial learning and memory. In the hidden platform version of the water maze, MOR KO mice show slower acquisition of the escape response and fail to match the performance of wild-type controls over 5 days of training. Additionally, they demonstrate degraded memory for the spatial location of the hidden platform when tested in a probe trial (Jang et al., 2003). MOR KO mice also are impaired in the spatial version of the eight-arm radial maze task, where they show retarded

acquisition and deficient asymptotic performance (Jamot et al., 2003). Both spatial learning and context fear conditioning depend critically on hippocampal function and the results of the present experiment are consistent with such dysfunction in our population of mice. In both the Jang and Jamot studies, MOR KO mice eventually learned the tasks (that is, they exhibited performance that was statistically better than chance performance). However, they were impaired when compared directly with their wild-type littermates. Unfortunately, those studies did not examine the heterozygous MOR KO mouse, so we cannot make any comparisons with our results with this genotype. Our homozygous and heterozygous MOR KO mice show significant learning in our fear conditioning task yet display subtle deficits when compared to wild-type controls. This deficit, if caused by dysfunction in the hippocampus, likely stems from a degraded representation of the training context. An impaired ability to form a context representation would be expected to lead to the deficit in the acquisition curve seen here. Site-directed MOR deletion would serve to confirm our suspicions about hippocampal involvement in the subtle context deficit presented here.

The results of the present experiments indicate that the heterozygous deletion of the MOR can have significant effects on some behaviors while leaving others relatively intact. Our results indicate that two copies of the MOR gene are necessary for normal fear conditioning but only one is necessary for normal morphine analgesia. Quantitative autoradiography indicates that heterozygous KO of the MOR gene results in 33–76% loss of receptor binding throughout brain areas where MOR normally are found (Kitchen et al., 1997). The most parsimonious explanation for the effects here is that the partial loss of MOR is sufficient to disrupt activity in the fear conditioning circuit but insufficient to disrupt activity in the hot-plate analgesia circuit. An additional dose–response study may reveal a pharmacological threshold below which a full complement of MOR is necessary for morphine analgesia. Alternatively, mechanisms of “receptor reserve” may differ between the circuits mediating fear conditioning and morphine analgesia. Sora and coworkers have shown that various behavioral effects of morphine are differentially impaired by heterozygous and homozygous deletion of the MOR. For instance, heterozygous animals show the same lack of morphine self-administration as homozygous animals. They show a level of morphine-induced locomotion intermediate between homozygous animals and wild-type controls. Finally, they resemble their wild-type littermates on tests of conditioned place preference and morphine analgesia (Sora et al., 2001). The results of Experiment 2 concerning morphine analgesia replicate these findings and add fear conditioning to the list of tasks on which a full complement of MOR appears necessary.

The deficit in context fear acquisition is surprising in light of our previous work on opioid receptor blockade, which implicated the MOR in fear conditional analgesia (Fanselow, 1981; Sanders & Fanselow, 2002). One possibility is that conditional analgesia in C57 mice may well prove to be mediated by other receptor subtypes, such as the delta receptor (Sanders & Fanselow, 2002). Another possibility is that MOR deletion affects conditional analgesia in a manner opposite to that produced by MOR blockade. Direct measures of fear conditional analgesia in MOR KO mice will aid in determining whether the phenotype revealed here is a result of alteration in the analgesia circuit. Additionally, we must consider that, in our line of animals, the MOR deletion is complete; it is present from conception and affects the entire organism. Consequently, we must entertain the possibility that the behavioral effects presented here (as well as those seen in the literature generally) are due to secondary effects either in the distribution of other opioid receptor populations or in entirely different neurotransmitter systems. The MOR deletion showed no secondary effects on other opioid receptor populations in binding studies using the whole brain (Matthes et al., 1996). Careful quantitative autoradiographic mapping has revealed subtle but significant alterations in delta receptor populations in MOR KO mice (Kitchen et al., 1997). Other studies have revealed that MOR deletion is accompanied by alterations in other neurotransmitter systems. MOR KO mice exhibit reduced D1/D2 mRNA expression (Park, Ho, Fan, Loh, & Ko, 2001) and reduced D2 receptor binding in the hippocampus (Matthes et al., 2000). MOR KO mice show increased NMDA receptor subunit mRNA in parietal cortex, hypothalamus, and thalamus, and also exhibit increased sensitivity to NMDA-induced convulsions (Jang, Lee, Loh, & Ho, 2001). Changes in these receptor systems could lead to alterations of both basic synaptic transmission and synaptic plasticity in the hippocampus as well as other structures. Comparisons of cued and context conditioning could serve to confirm the likely dysfunction of the hippocampus in the MOR KO mouse. Obviously, the MOR deletion does affect systems and structures that lie outside of our proposed negative feedback circuit mediating fear conditional analgesia. Inducible or targeted genetic deletion of the MOR could limit secondary effects and help considerably in elucidating the mechanism of the learning deficit seen in these global KO mice. In conclusion, our studies reveal that MOR deletion, unlike pharmacological MOR antagonism, causes a subtle deficit in Pavlovian fear conditioning. Future investigations of this deficit, under refined experimental procedures and employing inducible or targeted deletion, will illuminate further the role of MOR in fear learning.

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