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ORIGINAL INVESTIGATION

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# Delta opioid receptors: reflexive, defensive and vocal affective responses in female rats

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**Abstract** Ultrasonic vocalizations may be an expression of the affective pain response in laboratory animals. The present experiment compares the effects of morphine to the delta agonist, DPDPE (D-Pen$^2$, D-Pen$^5$ enkephalin) on a range of reflexive, behavioral and affective responses during an aggressive interaction. In experiment 1, naive female Long-Evans rats received morphine (0, 1, 3, 6, 10 μg ICV), or DPDPE (0, 30, 60, 100 μg ICV). In experiment 2, female rats were treated with naltrindole (1.0 mg/kg IP) 20 min before DPDPE (0, 60, 100 μg ICV). The following endpoints were measured: (1) latency to tail flick in response to heat stimuli; (2) high (33–65 kHz) and low (20–32 kHz) frequency ultrasonic and audible vocalizations; (3) defensive behavior; and (4) motoric activity. Following a brief exposure to attack, rats were threatened by the aggressor but protected from further attack by a large, wire mesh cage, thereby allowing for continued behavioral and vocal measurement without the risk of physical injury; video and audio recordings were made during the attack and then during a portion of the protected encounter (2 min). Morphine suppressed pain reactions varying in complexity from a spinal reflex, to an organized escape reaction, to an affective vocal response. The delta agonist, DPDPE, attenuated high frequency ultrasonic calling and tail flick responding. Defensive behaviors were also modulated by DPDPE at doses that had no effect on walking or rearing, indicating behavioral specificity. By contrast, doses of morphine that decreased defensive upright and escape also decreased motor activity. In female rats, morphine and DPDPE share a common profile of effects on a range of functional end-points, but DPDPE appears to modulate more selectively the reactions related to aversiveness without exerting sedative effects. These data demonstrate a possible physiological role for delta receptors in affective and defensive reactions.

**Key words** Ultrasound · Defense · Pain · Morphine · DPDPE · Naltrindole · Female · Aggression · Rats

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## Introduction

Opioid peptides and receptors play an integral role in the endogenous modulation of pain. Yet the analgesic efficacy of various opiate agonists differs as a function of the type of pain measured, as well as the specificity of the agonist for the mu, delta or kappa receptor subtype: kappa agonists inhibit pain induced by pressure, cold or the injection of acid (Schmauss and Yaksh 1984; Schmauss 1987). In rats, thermal pain is not effectively modulated by kappa agonists, but is attenuated by agents acting at the mu and delta receptors; although delta agonists are less potent thermal analgesics than mu agonists (Galligan et al. 1984; Cowan et al. 1988), selective antagonism (Portoghese et al. 1988; Calcagnetti et al. 1989; Heyman et al. 1989; Narita et al. 1993) and the absence of cross-tolerance (Kovacs et al. 1988; Suh and Tseng 1990) demonstrate that delta agonisits specifically influence sensitivity to thermal pain.

Which opioid receptor agonists modulate species-specific reactions to aversive *social* stimuli? Feline affective defense behavior is affected by both mu and delta receptor agonists (Shaikh et al. 1993). Morphine, predominantly a mu agonist, not only suppresses a spinal reflex to heat stimuli, but also modulates defensive behavior and ultrasonic calling, a possible affective vocal response to an aversive event (Hofer and Shair 1978; Tonoue et al. 1986; Cuomo et al. 1988). In rats, behavioral responses to painful or aversive stimuli are

often accompanied by ultrasonic vocalizations. Rat pups emit ultrasounds when separated from the dam (Sewell 1967), while adult rats ultrasound upon exposure to startling stimuli (Kaltwasser 1990; Van der Poel and Miczek 1991), and confrontation with an aggressive conspecific (Sales 1972), as well as to a range of other distressing events (see Miczek et al. 1995). More specifically, in situations of social conflict, male (Sales 1972, 1974; Lore et al. 1976; Takahashi et al. 1983) and female rats (Haney and Miczek 1993) emit both high (33–65 kHz) and low (20–32 kHz) frequency ultrasonic calls. High frequency calls, appear to be a measure of the affect of pain and fear: in mammals and birds, the mean frequency and the range of frequencies of individual vocalizations are elevated during fearful situations (Scherer 1986; Aubin and Bremond 1992). In addition, high frequency vocalizations are potently suppressed by morphine in both sexes (Vivian and Miczek 1993a; Haney and Miczek 1994) and by other pharmacological agents modifying fear, such as the benzodiazepines (Miczek et al. 1991; Shepherd et al. 1992; Vivian and Miczek 1993b; Tornatzky and Miczek 1995). Antagonism of the suppressive effect of morphine on high frequency ultrasounds by a low dose of naltrexone suggests mediation by the mu receptor (Vivian and Miczek 1993a; Haney and Miczek 1994). Yet it is not clear if these effects are *limited* to the mu receptor or if they also extend to other opiate receptor subtypes.

The present experiment compares the effects of morphine to the delta agonist, DPDPE (D-Pen$^2$, D-Pen$^5$ enkephalin) on a range of reflexive, behavioral and affective responses during an aggressive interaction. DPDPE, which specifically modulates thermal pain, was used in order to expand upon previous findings with morphine. An additional objective was to characterize the function of the delta receptor. Although the effects of DPDPE on tail flick reactions have already been investigated in male rats, there is little information on the role of the delta receptor in ultrasonic vocalizations and defensive responding during an aversive social interaction in either males or females. The first experiment compares morphine and DPDPE, while the second experiment investigates the specificity of DPDPE using the delta receptor antagonist, naltrindole (Portoghese et al. 1988).

## Materials and methods

### Subjects

Female Long-Evans rats (Charles River Laboratory, Wilmington Mass.), weighing between 230 and 300 g, were individually housed in polycarbonate cages (48 × 27 × 20 cm) with wood-chip bedding. Purina Rodent chow and water were freely available. The environment was maintained at 22 + 1°C with 30–40% humidity. Experiments were conducted between 1000 and 1330 hours, during the dark period of a 12-h (2000:0800) light-dark cycle in order to control for circadian changes in opiate binding and sensitivity (Bornschein et al. 1977; Kafka et al. 1983). In experiment 1, the estrous cycle of the cannulated rats was monitored daily by recording the occurrence of leukocytes and nucleated or cornified epithelial cells. Previous work has shown that a range of vocal and behavioral measures are influenced by the estrous cycle (Haney and Miczek 1994). In order to minimize this effect, animals were tested in different phases of the estrous cycle. In the subset of animals that demonstrated regular 4- to 5- day cycles, 11 were in estrus, 7 were in metestrus, 10 were in diestrus, and 9 were in proestrus on the day of drug administration. The remaining animals were acyclic.

### Surgery

For each procedure, care was taken to minimize discomfort to the rats. Subjects were handled daily, before and after surgery, to decrease the stress of the experimental procedures. Rats received ketamine hydrochloride (75 mg/kg intraperitoneal) and 5 min later, xylazine (6 mg/kg intramuscular) and penicillin (Ambi-pen: 1 ml subcutaneous). A 23 ga cannula was implanted into the left cerebral ventrical (bregma: posterior −0.8 mm; lateral 1.6 mm; ventral 2.7 mm from brain surface) (Paxinos and Watson 1986) and secured to the skull with dental cement. The incisor bar was set at 3.3 mm below interaural line. A 7 mm (30 ga) obdurator was placed in the guide cannula to keep it clear.

### Drug administration

DPDPE and morphine were dissolved in distilled water (pH 6.7). Drugs were infused into the ventricle by backloading an injector cannula (30 ga) that was connected to a 25-ml Hamilton, syringe (Hamilton, Reno, Mev.) with polyethylene tubing (PE-50); injector cannulae extended 0.5–1.0 mm beyond the tip of the guide cannula. A syringe pump (Sage Instruments, model 341, Cambridge, Mass.) infused solution (5–10 ml) over a 2- to 3-min interval. Animals were free to move around the immediate area surrounding the pump. Injector cannulae were left in place for at least 1 min after the infusion and animals were returned to the home cage (Myers 1971). In experiment 1, DPDPE (0, 30, 60, 100 μg) and morphine (0, 1, 3, 6, 10 μg) were used, while in experiment 2, naltrindole (1.0 mg/kg IP) was dissolved in distilled water and injected 20 min prior to DPDPE (0, 60, 100 μg).

### Experimental schedule

Animals were given at least a week to recover from the surgery before encountering an aggressive conspecific. On the day of testing, rats in their home-cage were placed into the photocell apparatus. Baseline tail flick latency was determined, and distilled water, DPDPE or morphine was infused; each animal received only one infusion. Tail flick latency was measured every 10–20 min after infusion for 100 min; locomotor activity within the home cage was measured for 5 min, 25 min post-infusion. Thirty minutes post-infusion, rats were placed into the home-cage of a lactating female that had previously demonstrated high levels of maternal aggression (criteria: 20 attack bites within 2 min), i.e. the "attack encounter". The pups and the male cage-mate of the lactating female were removed prior to introduction of the experimental animal. The aggressive interaction was terminated once the intruder had either (1) received 20 attack bites or was (2) consecutively supine for 3 s.

Following the attack encounter, rats were then placed into a clean, protective cage, constructed of wire mesh on all sides (30 × 21.5 × 20 cm), and returned to the home-cage of the lactating female for 60 min, i.e. the "threat of attack". This cage did not constrain the animals, but was large enough for them to walk, rear and groom. During the protected encounter, lactating females continue to threaten and attack the experimental rat in the protective cage

(Haney and Miczek 1993). Although the intensity of the confrontation is lower than during the attack encounter, based on endocrine, cardiovascular and thermoregulatory measures (Miczek et al. 1995), rats in the protective cage continue to emit high and low frequency ultrasounds. At 40 and 60 min post-infusion, audio and video tape-recordings of the interaction were made (2 min), followed by a measure of tail flick latency (Table 1).

Animals were killed by an overdose of ketamine (150 mg/kg IP) and 1% toluidine blue (5 µl) was infused into the cannula, to verify macroscopically its position in the ventricle. To be certain that each animal received drugs into the ventricle, only those that met at least two of the following three criteria were included in the analysis: (1) capillary flow: a cannula backloaded with distilled water was inserted and the movement of distilled water was monitored. The liquid flows freely if the cannula is in the ventricle but does not move if it is in tissue; (2) angiotensin-induced drinking: cannula patency was checked 1–3 days after surgery by monitoring the effects of angiotensin II (25 ng/5 ml ICV) on drinking behavior. Cannula placement was considered accurate if animals drank at least 5 ml water within 15 min; (3) dye infusion: only animals that had toluidine blue in the lateral, third and fourth ventricles were included in the subsequent statistical analysis. A total of 16 animals from the first and second experiments were not included in the analysis for not achieving two out of three of these criteria.

### Assessment of locomotor activity

Ambulatory and non-ambulatory activity within the home-cage was measured with the Opto-Varimex Mini (Columbus Instruments, Columbus, Ohio). Infrared light beams surrounded the perimeter of the home cage. Ambulatory movement was defined as the number of times an animal broke a new beam of infrared light. Repeatedly breaking the same beam of light was considered non-ambulatory movement, such as grooming or rearing.

### Ultrasound measurement

Ultrasounds were recorded with a condensor microphone (Bruel and Kjaer type 4135, Naerum, Denmark), preamplifier (Bruel and Kjaer type 2633), amplifier (Bruel and Kjaer type 2610), and filter (Krohn-Hite model 3550R: high pass: 10 kHz, low pass: 100 kHz). During the experiment, ultrasounds were directly monitored by oscilloscope and recorded onto audio tapes (Maxell UD 25–120N) using an eight-channel instrumentation recorder (Hewlett-Packard, Model 3968A, Santa Clara CA) (Vivian and Miczek 1991). Low (20–32 kHz) and high (32–65 kHz) frequency ultrasounds and audible sounds were analyzed by playing the tape at one quarter speed through an amplifier into headphones. Listeners were trained to press a key in response to each type of call, thereby measuring its rate and duration. Individual low frequency calls often contain high frequency components at the beginning and/or the end of the call (Kaltwasser 1990; Haney and Miczek 1993). During the audio scoring of ultrasounds, these calls were considered low frequency ultrasounds because the medial segment was predominantly monotonous and of a relatively long duration. A call was considered high frequency if it was not continuous with the preceding or proceeding ultrasound but occurred independently. Vocalization data are expressed in terms of rate (number of calls/min).

### Assessment of agonistic behavior

Behavioral interactions were recorded with an infrared-sensitive camera (Canon Model Ci20R) and a VHS video cassette recorder (Zenith, Model VR 3300, Glenview, Ill. Videorecords of the resident and the intruder during the aggressive and protected encounters (2 min) were evaluated in terms of the frequency and duration of specific acts and posture, using customized software (Miczek 1982). Each behavioral item was encoded by the depression of a designated key on a hand-held console when the behavior started and the release of the key when it stopped. The video cassette recorder had a time-code generator allowing for behavioral observation in slow motion. Female intruders exhibited escape (fleeing the resident), defensive upright (standing erect with forepaws extended), crouching (four paws on ground, not orienting towards resident), and supine (forced on back by the aggressive posture of the resident) postures, as well as walking, rearing, grooming, digging (Haney and Miczek 1993). During the phase of the encounter when the intruder was protected from attack, nasal contact between the resident and intruder, crouching, walking, rearing and grooming were measured. In the attack encounter, the duration of each interaction varied between individuals, so that the percentage of time spent in each behavior relative to the total duration of the interaction was analyzed.

### Assessment of pain sensitivity

Tail flick latency in reaction to a heat stimulus (EMDE, Richmond, Va.) was used to measure pain sensitivity (D'Amour and Smith 1941). The light intensity used generated control latencies between 1.7 and 3.0 s. The light beam was focused on the rat's tail and the median of three consecutive latencies was recorded. If latency exceeded 6.0 s the heat stimulus was automatically terminated in order to prevent tissue damage.

### Statistical analysis

Experiment 1 was analyzed using a one-way ANOVA (dose), while in experiment 2, one-way ANOVA (DPDPE dose) in the presence or in the absence of naltrindole were compared. Post-hoc analyses included the Dunnett's *t*-test to compare individual doses to control and the Tukey-Kramer statistic for comparing the same dose of DPDPE in the presence or absence of naltrindole. Occasional missing data points occurred due to equipment failure.

Tail flick data were converted to the percent maximal possible effect (%MPE) by comparing the measured change in tail flick latency to the maximal change in tail flick latency (Dewey and Harris 1975). Animals that did not respond within 6.0 s were considered 100% analgesic.

**Table 1** Experiment 1 time line. Morphine or DPDPE were administered intracerebrobentricularly (*ICV*). Attack encounter refers to the interaction between the intruder and the lactating resident female. The protected cage is where the intruder is in the presence of the resident but protected from attack by wire mesh

| t0 | t10 | t20 | t25 | t30 | t40 | t60 |
|---|---|---|---|---|---|---|
| Baseline tailflick<br>Inject (ICV)<br>Return to home cage | Tail flick | Tail flick | Activity measure<br>(5 min) | Attack encounter<br>Video- and audio-tape<br>Tail flick<br>Protected cage | Video- and audio-tape<br>(2 min)<br>Tail flick | Video- and audio-tape<br>(2 min)<br>Tail flick |

$$\% \text{ MPE} = \frac{\text{Post-drug latency} - \text{Baseline latency}}{\text{Cut-off latency} - \text{Baseline latency}} \times 100$$

These and other data expressed as percentages do not meet the homogeneity of variance assumption of parametric statistics and were therefore transformed (arc sine-square root transformation) prior to the ANOVA.

## Results

### Locomotor activity

*Experiment 1*

DPDPE (30 µg) increased horizontal locomotor activity, i.e. walking within the home cage [$F(3, 40) = 4.04$, $P < 0.01$: 30 µg, $t_D = P < 0.05$]. Morphine significantly suppressed locomotion [$F(3, 41) = 5.20$, $P < 0.01$: 10 µg, $t_D = P < 0.05$].

*Experiment 2*

DPDPE significantly altered horizontal locomotor activity: [$F(2, 15) = 4.27$, $P < 0.05$]. Rats tended to be more active at 60 µg and less active at 100 µg, in relation to placebo. Naltrindole did not alter this pattern of effects.

### Vocalizations

*Experiment 1*

DPDPE selectively attenuated high frequency ultrasounds (33–65 kHz) measured during either the attack encounter [$F(3, 44) = 4.84$, $P < 0.01$; 60, 100 µg, $t_D = P < 0.05$] or the threat of attack [40 min post-infusion: $F(3, 43) = 5.18$, $P < 0.01$; 30, 60, 100 µg, $t_D = P < 0.05$]; DPDPE had no significant effect on ultrasounds 1 h post-infusion. Morphine suppressed audible vocalizations [$F(4, 48) = 2.95$, $P < 0.05$], as well as high [$F(4, 48) = 4.33$, $P < 0.01$; 6, 10 µg, $t_D = P < 0.05$] and low frequency ultrasounds [$F(4, 48) = 4.34$, $P < 0.01$; 6, 10 µg, $t_D = P < 0.05$] during the attack encounter. Morphine also attenuated high frequency ultrasounds emitted in the protected cage, at 40 [$F(4, 47) = 6.36$, $P < 0.01$; 1, 3, 6, 10 µg, $t_D = P < 0.05$] but not 60 min post-infusion.

*Experiment 2*

Similar to experiment 1, DPDPE attenuated high frequency ultrasounds during the attack encounter [$F(2, 15) = 8.21$, $P < 0.01$; 60, 100 µg, $t_D = P < 0.05$] and the threat of attack [40 min post-infusion: $F(2, 15) = 7.23$, $P < 0.05$; 60, 100 µg, $t_D = P < 0.05$].

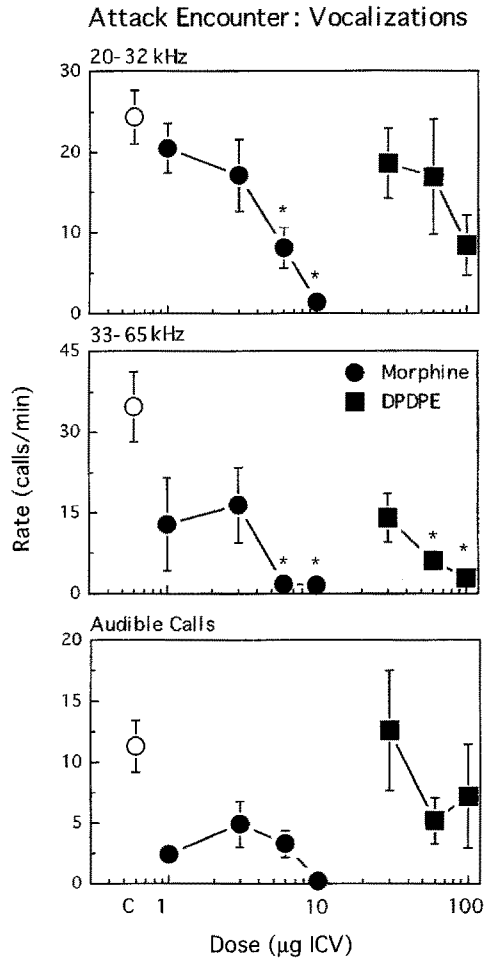


**Fig. 1** Effect of morphine and DPDPE on low frequency (20–32 kHz) and high frequency (33–65 kHz) ultrasonic calling, and audible vocalizations during direct contact with an aggressive lactating female. Testing occurred 30 min post-injection. Each dose represents 5–10 rats. Controls (*C*) received $dH_20$ ($n = 24$). Error bars represent SEM. *Asterisks* denote a significant difference from control, $^*P < 0.05$

DPDPE also attenuated low frequency ultrasounds emitted during the attack [$F(2, 15) = 5.97$, $P < 0.01$; 60, 100 µg, $t_D = P < 0.05$], but not during the protected encounters. One hour post-infusion, the highest dose of DPDPE continued to suppress high frequency calls [60 min post-infusion: $F(2,15) = 3.56$, $P < 0.05$; 100 µg, $t_D = P < 0.05$]. Naltrindole given in the absence of DPDPE (0 µg) significantly decreased high and low frequency ultrasounds during the attack encounter, compared to the control injection without naltrindole (TK = $P < 0.05$). The suppressive effects of naltrindole alone precluded any effect of DPDPE on vocalizations during the attack encounter. During the threat of attack, DPDPE's suppressive effects on high frequency ultrasounds were not significant in the presence of naltrindole, either 40 min ($P < 0.14$) or 60 min post-infusion ($P < 0.63$), presumably reflecting naltrindole's tendency to decrease ultrasound calling rate under control conditions.

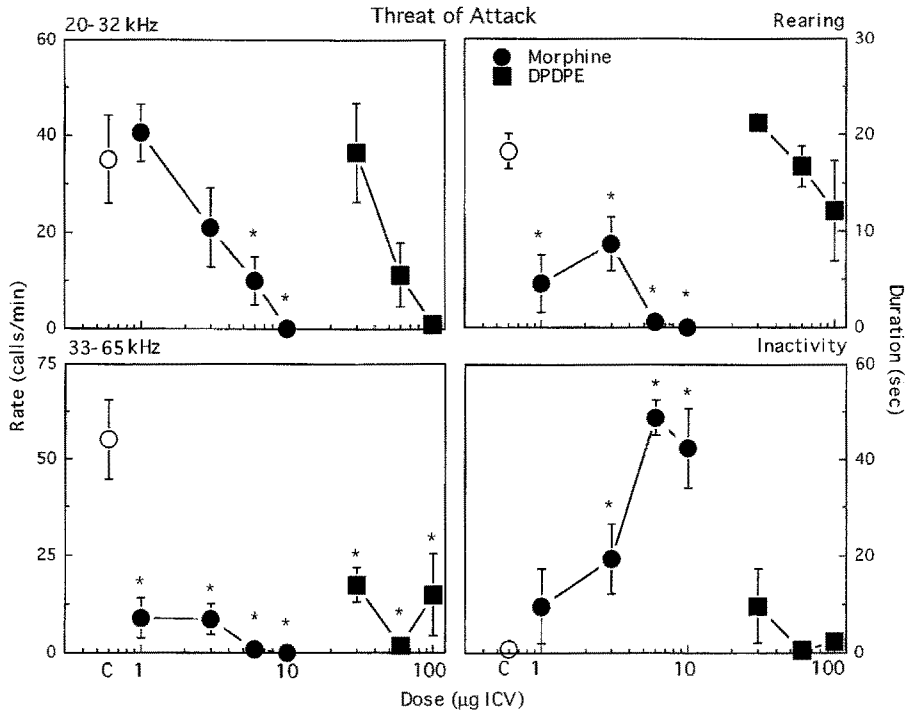


**Fig. 2** Effect of morphine or DPDPE during the threat of attack, where animals were threatened by but protected from the attacker by a wire mesh cage. Testing (2 min) occurred 40 min post-injection. Motoric activity (duration/min), low frequency (20–32 kHz) and high frequency (33–65 kHz) ultrasonic calls were measured. Each dose represents 5–10 rats. Controls (*C*) received $dH_20$ ($n = 24$). Error bars represent SEM. *Asterisks* denote a significant difference from control, $^*P < 0.05$

Tail flick latency

*Experiment 1*

Both DPDPE [30 min post-infusion: $F(3, 42) = 9.94$, $P < 0.01$; 60,100 µg, $t_D = P < 0.05$; 40 min post-infusion: $F(3, 41) = 3.05$, $P < 0.05$; 60 µg, $t_D = P < 0.05$] and morphine significantly increased tail flick latency [30 min post-infusion: $F(4, 46) = 17.29$, $P < 0.01$; 6, 10 µg, $t_D = P < 0.05$; 40 min post-infusion: $F(4, 46) = 13.69$, $P < 0.01$; 6, 10 $t_D = P < 0.05$].

*Experiment 2*

DPDPE [30 min post-infusion: $F(2,14) = 28.78$, $P < 0.001$; 100 µg, $t_D = P < 0.05$; 40 min post-infusion: $F(2, 14) = 87.50$, $P < 0.001$; 100 µg, $t_D = P < 0.05$] significantly increased tail flick latency. Naltrindole significantly reduced analgesia following DPDPE (100 µg) administration (30 min post-infusion: TK = $P < 0.05$; 40 min post-infusion: TK = $P < 0.05$).

Defensive behavior: attack encounter

*Experiment 1*

Female rats in the control group spent close to half (47.8%) of the attack encounter in a defensive upright posture. The other defensive behaviors displayed were crouching (10.1%), escape (7.6%) and supine (7.8%) postures. Both morphine [$F(4, 46) = 12.67$, $P < 0.01$: 1, 6, 10 µg, $t_D = P < 0.05$] and DPDPE [$F(3, 43) = 7.93$, $P < 0.01$: 30, 60, 100 µg, $t_D = P < 0.05$] decreased the amount of time spent in a defensive upright, while increasing the duration of crouching [morphine [$F(4, 46) = 7.70$, $P < 0.01$: 6, 10 µg, $T_D = 2.58$, $P < 0.05$]; DPDPE: [$F(3, 43) = 7.26$, $P < 0.01$: 60, 100 µg, $t_D = 2.47$, $P < 0.05$)]. Morphine also significantly decreased escape behavior [$F(4, 46) = 4.70$, $P < 0.01$: 6, 10 µg, $t_D = P < 0.05$]. The only motor behavior occurring for any appreciable length of time in the presence of the resident was walking. Morphine significantly increased walking at the lowest dose [$F(4, 46) = 3.45$, $P < 0.01$: 1 µg, $t_D = P < 0.05$] and decreased walking at the highest dose; DPDPE did not significantly modulate walking.

*Experiment 2*

DPDPE decreased the amount of time spent in a defensive upright posture [$F(2, 15) = 7.56$, $P < 0.01$: 60, 100 µg, $t_D = P < 0.05$]. Naltrindole did not reverse this effect. DPDPE also decreased walking [$F(2, 15) = 3.98$, $P < 0.05$: 100 µg, $t_D = P < 0.05$]. In the presence of naltrindole, DPDPE did not significantly modulate walking, due to the tendency for naltrindole alone to decrease walking ($P < 0.11$).

Defensive behavior: protected encounter

*Experiment 1*

Ten minutes after being attacked and placed into the protected cage, control animals spent most of the time

**Table 2** DPDPE and Naltrindole Means and SEM of low (20–32 KHz) and high frequency (33–65 KHz) ultrasounds, expressed as the rate of calls/min, and behavior (s/min) during the threat of attack. Attack refers to a direct confrontation with an aggressive lactating female (30 min post-injection). Threat refers to exposure to the attacking animal but protection by a wire mesh cage (40 min post-injection). Each dose represents six rats except for the control dose without naltrindole ($n = 5$).

| Measure | DPDPE (μg) | | | DPDPE (μg) + naltrindole (1.0 mg/kg) | | |
|---|---|---|---|---|---|---|
| | 0.0 | 60.0 | 100.0 | 0.0 | 60.0 | 100.0 |
| *Attack* | | | | | | |
| Low (20–32 kHz) | 62.1 | 2.9* | 8.2* | 30.5# | 25.5 | 13.2 |
| | 22.7 | 2.2 | 5.4 | 5.8 | 7.4 | 5.8 |
| High (33–65 kHz) | 52.8 | 2.9* | 0.3* | 5.4# | 1.1 | 1.7 |
| | 17.8 | 2.1 | 0.3 | 2.1 | 0.9 | 0.7 |
| *Threat of attack* | | | | | | |
| Low (20–32 kHz) | 33.7 | 83.9 | 5.2 | 56.8 | 94.2 | 10.8 |
| | 14.1 | 23.2 | 3.5 | 14.1 | 28.4 | 6.2 |
| High (33–65 kHz) | 45.3 | 17.0* | 0.2* | 25.7 | 5.2 | 0.6 |
| | 12.7 | 7.3 | 0.1 | 16.8 | 2.8 | 0.2 |
| Inactivity | 7.2 | 1.8 | 38.0 | 1.8 | 0.6 | 28.4 |
| | 4.0 | 0.7 | 9.6 | 1.3 | 0.4 | 11.4 |
| Rearing | 12.9 | 17.2 | 3.1 | 13.4 | 24.6 | 2.9 |
| | 3.1 | 3.1 | 2.8 | 2.3 | 3.4 | 2.0 |

*Asterisks* indicate significantly different from control. *Number* symbols indicate significantly different from control without naltrindole ($P < 0.05$)

walking, rearing and occasionally gnawing on the walls of the cage. The primary social contact between the resident and the intruder was nasal contact through the wire mesh; nasal contact was often followed by the resident attempting to attack the intruder through the wire mesh screen.

DPDPE and morphine had a considerably different profile of effects on activity within the protected cage (Fig. 2). Morphine significantly suppressed all motor activity: [rearing $F(4, 47) = 13.78$, $P < 0.01$: 1, 3, 6, 10 μg, $t_D = P < 0.01$; walking $F(4, 47) = 10.83$, $P < 0.01$: 1, 3, 6 μg, $t_D = P < 0.05$; and chewing the protective cage $F(4, 48) = 3.83$, $P < 0.01$: 6, 10 μg, $t_D = P < 0.05$], while inactivity was dose-dependently increased [$F(4, 46) = 29.73$, $P < 0.01$: 3, 6, 10 μg, $t_D = P < 0.05$]. By contrast, DPDPE had no effect on the amount of time spent in any of these activities; animals continued to walk, rear, groom, chew the cage and make contact with the resident through the cage.

The pattern of motor activity changed 30 min after the female had been in the presence of the attacker but protected from attack (60 min post-infusion). Although a similar proportion of time was spent walking, control animals reared less and spent more time grooming or remaining inactive than 40 min post-infusion. DPDPE had no effect at this time point, while morphine continued to suppress motor activity. Grooming [$F(4, 44) = 2.62$, $P < 0.05$: 6, 10 μg, $t_D = P < 0.05$] and walking duration [$F(4, 40) = 3.63$, $P < 0.01$: 10 μg, $t_D = P < 0.05$] were decreased by morphine, while inactivity was increased [$F(4, 39) = 7.70$, $P < 0.01$: 6, 10 μg, $t_D = P < 0.05$].

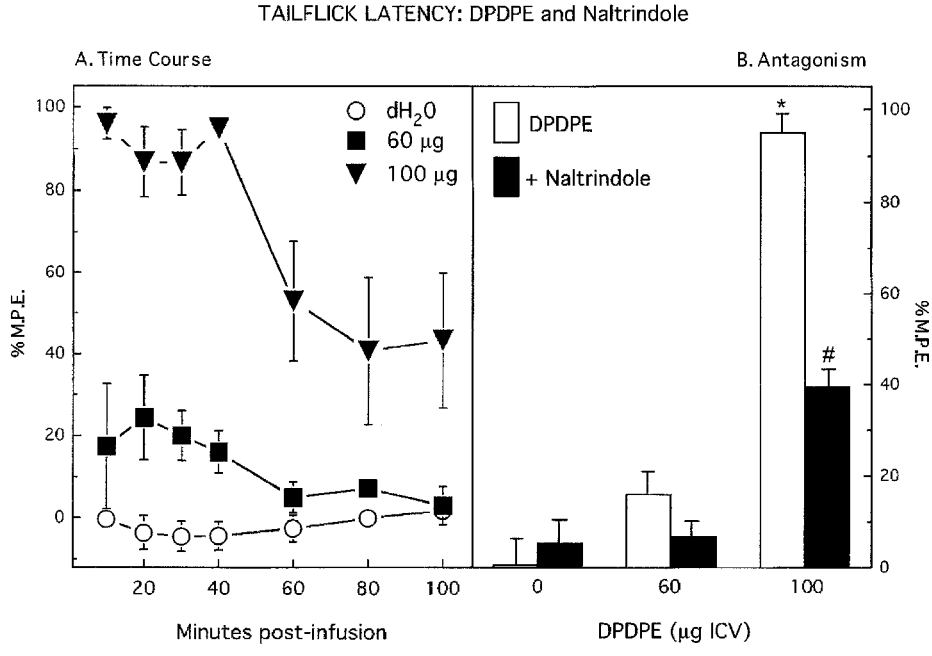


**Fig. 3** **A** Time course of the effect of DPDPE on the latency to tail flick in response to heat stimuli, expressed as the maximal possible effect (% MPE). **B** Effect of naltrindole (1.0 mg/kg) 40 min following DPDPE administration. Controls received $dH_20$. There were six rats/dose except for the control group without naltrindole ($n = 5$). *Asterisks* denote a significant difference from control ($*P < 0.05$). *Number* symbol signifies significantly different from DPDPE alone ($\#P < 0.05$)

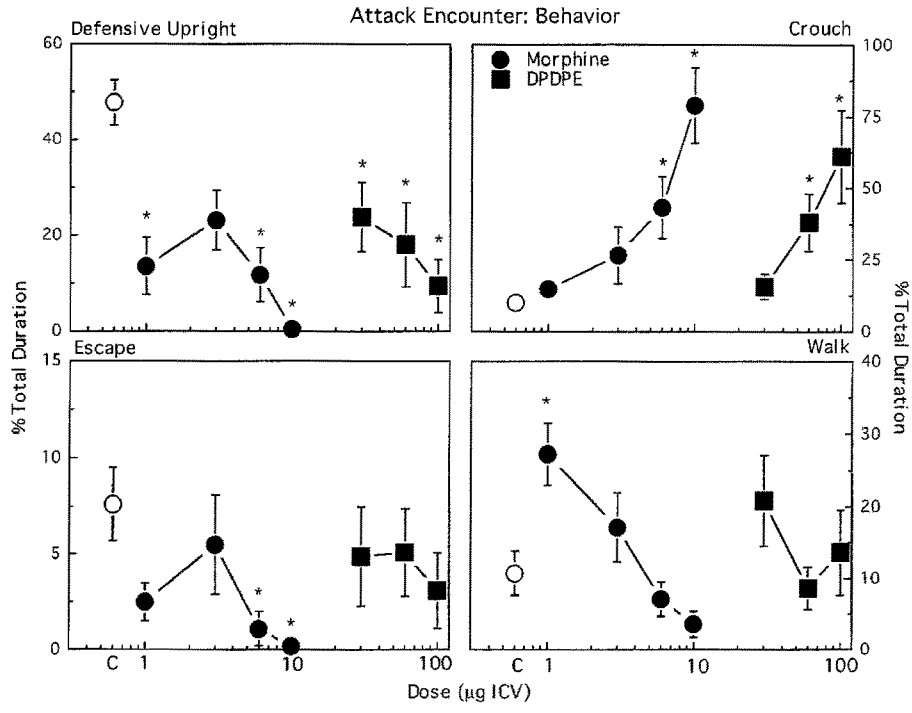


**Fig. 4** Effect of morphine or DPDPE on defensive and locomotor behavior during the attack encounter, where animals were in direct contact with an aggressive lactating female. Proportion of time spent in each type of behavior in relation to the total time in the presence of the attacker is presented. Testing occurred 30 min post-injection. *Asterisks* denote a significant difference from saline control, $*P < 0.05$. Error bars represent SEM

*Experiment 2*

At 40 min post-infusion, the highest dose of DPDPE decreased rearing [$F(2, 15) = 5.69$, $P < 0.05$] while increasing immobility [$F(2, 15) = 10.48$, $P < 0.01$: 100 μg, $t_D = P < 0.05$); other behaviors were not significantly affected by DPDPE. DPDPE had no significant behavioral effects 60 min post-infusion. Naltrindole did not alter this pattern of effects (Table 2).

## Discussion

The role of delta and mu receptors in the response to aversive stimuli can be dissociated when a range of specific and non-specific behavioral responses are compared within the same animal. For morphine, the profile of effects following central administration closely parallels the findings obtained with systemic administration (Haney and Miczek 1994): intraventricular morphine suppressed pain reactions varying in complexity from a spinal reflex, to an organized escape reaction, to an affective vocal response. The delta agonist, DPDPE, attenuated high frequency ultrasonic calling and tail flick responding. Defensive behaviors were also specifically modulated by DPDPE at doses that had no effect on walking or rearing. By contrast, doses of morphine that decreased defensive upright and escape also decreased motor activity. This pattern suggests that in female rats, DPDPE is less potent than morphine, but is more selective in modulating reactions specific to aversive stimuli, i.e. ultrasonic calling, defensive upright posture, and crouching, without compromising motoric activity in general.

One way to understand these differences between DPDPE and morphine is to compare the neuroanatomical localization of the delta and mu receptor. Delta receptors are concentrated in forebrain structures, i.e., olfactory bulb and tubercle, n. accumbens, basolateral amygdala, claustrum, and caudate-putamen, while binding is generally low throughout the diencephalon, mesencephalon and pons/medulla. Mu receptors are also highly concentrated in the n. accumbens and caudate-putamen, and in limbic structures, such as the amygdala. Unlike delta receptors, mu receptors are found in high densities in the thalamus and in most midbrain and brainstem sites essential to pain modulation, such as the raphe nuclei and the periaqueductal gray (Mansour et al. 1988; Sharif and Hughes 1989; Fowler and Fraser 1994).

The potency with which both morphine and DPDPE suppressed high frequency vocalizations may reflect binding in limbic sites. Nonspecific explanations for opiate-induced ultrasound suppression i.e., thermoregulatory or respiratory effects, appear unlikely, since delta agonists do not share these properties (Sharif and Hughes 1989; Handler et al. 1992). Yet the amygdala, which is integral to the production of vocalizations across a range of species (Siegel and Pott 1988, Hammond 1989); is (1) rich in mu and delta receptors (Mansour et al. 1988), (2) projects to midbrain sites mediating both vocalizations (Jurgens and Pratt 1979; Wetzel et al. 1980) and affective defensive behaviors (Shaikh et al. 1993), and (3) modulates the expression of higher order pain reactions (Schmauss and Yaksh 1984; Schmauss 1987). Low frequency ultrasounds in response to attacks and threats do not appear to be specifically modulated by benzodiazepine or opiate

agonists (Shepherd et al. 1992; Vivian and Miczek 1993b). We have previously suggested that high frequency ultrasounds are a more sensitive measure of the affective response to an attacking conspecific (Haney and Miczek 1994). If so, the present data suggest that both mu and delta agonists can attenuate an affective component to pain.

The different motoric properties of DPDPE and morphine are most likely due to the distinct neurochemical effects of mu and delta agonists at the level of the n. accumbens and caudate nucleus (Vezina et al. 1987; Dauge et al. 1988, 1989). DPDPE and morphine exerted opposite effects on motor activity while animals were in their home cage. The lowest dose of DPDPE tripled measures of ambulatory movement, while morphine was dose-dependently suppressive. During the threat of attack, morphine inhibited both walking and rearing, while DPDPE did not; DPDPE was still potently suppressing high frequency vocalizations at this time-point, so the absence of motor inhibition did not reflect drug metabolism.

The potent suppression of high frequency ultrasounds after the intruder received either naltrindole or DPDPE does, however, provide further indication that the attacking resident is not the source of the calls, as ultrasounds were suppressed at doses that did not modify agonistic or motoric behavior. More direct evidence is that devocalizing male intruders eliminates both high and low frequency calls, whereas devocalizing the resident does not (Takahashi et al. 1983; Thomas et al. 1983). In addition, simultaneous calling was not detected on the audio recordings, which would have occurred if the resident was also emitting ultrasounds.

Previously, morphine's effects on high frequency ultrasonic vocalizations, tail flick latency and motoric behavior were shown to be attenuated by naltrexone pretreatment (Haney and Miczek 1994). In the present experiment, the specificity of DPDPE was investigated, using the antagonist, naltrindole. Naltrindole antagonized analgesia following DPDPE administration, and blocked DPDPE from significantly decreasing ultrasounds and walking during the attack encounter. However, these behavioral effects were not entirely due to an antagonism but rather to naltrindole's intrinsic properties, i.e. decreased walking, and ultrasound calling rate. These effects do not appear to reflect the displacement of an endogenous delta receptor ligand, because they were similar to the delta receptor agonist, DPDPE: the threat of attack *increased* vocalizations, while both the agonist and the antagonist decreased vocalizations. Other evidence points to a possible partial agonist activity by naltrindole. In male rats, naltrindole (10 mg) produced a small increase in tail immersion latency (Jackson et al. 1989). Naltrindole alone had intrinsic effects that make it difficult to address the question of DPDPE receptor specificity.

In summary, the present results contribute to the understanding of delta receptor function by providing the first evidence of specific delta agonist attenuation of ultrasounds emitted during an aggressive encounter, i.e. an effect on a potentially affective response. Morphine and DPDPE generate a common profile of effects on a range of end-points, but DPDPE appears to more selectively modulate the reactions related to aversiveness without exerting any sedative effects in female rats. These data demonstrate a possible physiological role for delta receptors in affective and defensive reactions.

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