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## Pain and negative affect: evidence the inverse benzodiazepine agonist DMCM inhibits pain and learning in rats

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**Abstract** *Rationale:* The anxiogenic DMCM, an inverse benzodiazepine agonist, was used to explore the relationship between negative affective states and pain. Past work suggests that the outcome obtained may depend on both the intensity of the affective state and the way in which pain is inferred. *Objectives:* The present study was designed to test the impact of relatively low doses of DMCM on multiple measures of pain reactivity and learning. *Methods:* In experiment 1, systemic injections of 0.00, 0.015, 0.06, and 0.25 mg/kg DMCM were administered before vocalization and tail movements were assessed in response to a gradually incremented shock and radiant heat stimulus. Experiment 2 tested the effects of DMCM on Pavlovian conditioning. DMCM-treated subjects experienced a context paired with an aversive unconditioned stimulus (US) and conditioned freezing was assessed the next day. *Results:* Experiment 1 showed that DMCM inhibits both a spinal nociceptive reflex (tail-flick to heat) and a supraspinal measure of pain (vocalization to shock). Because these inhibitory effects could reflect a disruption in motor function, experiment 2 employed a remote test based on Pavlovian conditioning. A moderate dose of DMCM undermined learning, implying that the drug decreased the affective impact of the aversive US. *Conclusions:* DMCM induces hypoalgesia on a wide range of assays. Furthermore, pharmacologically inducing a negative affective state blocks Pavlovian fear conditioning. It is suggested that DMCM induces a state of panic and that this state inhibits pain.

**Keywords** Pain · Pavlovian conditioning · DMCM · Anxiety · Fear · Panic

### Introduction

Intuitively, it seems obvious that negative affective states influence pain and learning. Yet, theory and data suggest opposing views on the directionality of that modification (Bolles and Fanselow 1980; Terman et al. 1984; Fanselow 1986, 1994; King et al. 1996; Rhudy and Meagher 2000). One approach, the perceptual-defensive-recuperative (PDR) model originally proposed by Bolles and Fanselow (1980) and subsequently updated by Fanselow (1986, 1994), assumes fear inhibits pain and, as a consequence, undermines learning about aversive stimuli. According to these authors, fear and pain represent distinct motivational systems. An aversive stimulus can not only engage the pain motivational system, but can also act as an unconditioned stimulus (US) and support Pavlovian fear conditioning. According to Bolles and Fanselow (1980), a cue (the conditioned stimulus, or CS) that predicts the US elicits an expectation of pain and this in turn generates fear. It is then assumed that engagement of this fear motivational system raises pain thresholds (hypoalgesia) through brainstem systems that inhibit the flow of incoming nociceptive (pain-related) signals. This antinociception undermines the painfulness of subsequent aversive stimuli, and thereby, reduces their ability to support new learning. Supporting this, researchers have shown that a CS previously paired with shock reduces nociceptive reactivity on some standard measures of pain: tail withdrawal to radiant heat (the tail-flick test) and recuperative behavior directed toward a paw injected with a mild irritant (the formalin test) (Chance et al. 1977; Fanselow and Baackes 1982; Watkins et al. 1982). Moreover, this conditioned hypoalgesia is attenuated by manipulations that reduce conditioned fear (Fanselow 1986; Helmstetter and Bellgowan 1993) and presenting a CS that elicits fear appears to reduce the effectiveness of the US and undermine learning (Fanselow and Bolles 1979).

A very different perspective has been suggested by researchers working in the human pain literature, where it has been argued that negative affective states enhance pain (Haslam 1966; Bowers 1968; Dougher 1979;

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Schumacher and Velden 1984; Weisenberg et al. 1984; Dougher et al. 1987; Cornwall and Donderi 1988; Al Absi and Rokke 1991; Rhudy and Meagher 2000). The most popular version of this hypothesis assumes anxiety facilitates pain, an observation that has led to the development of relaxation techniques to reduce pain in clinical settings (see, for example, Turk et al. 1983). Anxiety is often characterized as a state akin to fear that is elicited under conditions of uncertainty, a form of anxious apprehension associated with a lack of control and/or unpredictability (Barlow et al. 1996). In contrast, Bolles and Fanselow (1980) and others (Fanselow 1994; Barlow et al. 1996) characterize fear as a state elicited by identifiable cues within the environment, cues that innately or through learning predict imminent danger and inhibit pain. While Bolles and Fanselow (1980) assume fear inhibits pain, they acknowledge that pain could be enhanced by a state that was of "the prolonged-duration, ill-defined variety usually called anxiety" (p 299).

If anxiety enhances pain, then pharmacologically inducing anxiety should also lower pain thresholds and facilitate learning. Past work, however, has yielded ambiguous and/or contradictory results (Rodgers and Randall 1987a; Helmsetter et al. 1990; Fanselow et al. 1991; Fanselow and Kim 1992). The most powerful anxiogenics are the benzodiazepine inverse agonists, drugs such as methyl-6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate (DMCM) and N-methyl-beta-carboline-3-carboximide (FG 7142) (Dorow et al. 1983; Fanselow et al. 1991). In humans, administration of FG 7142 has been shown to elicit feelings of severe anxiety and an "...fear of death and annihilation" accompanied by symptoms of "inner tension", trembling, profuse sweating, increased respiration and heart rate, as well as increased cortisol levels in the blood (Dorow et al. 1983). If DMCM and FG 7142 induce a state of enhanced pain (hyperalgesia), the drugs should increase the affective impact of aversive stimuli in a learning task. Supporting this, low doses of DMCM and/or FG 7142 appear to enhance the suppressive effects of a weak shock in a punishment paradigm and facilitate learning in a passive avoidance task (File and Pellow 1988; Boer et al. 1992). Yet, other studies suggest that these drugs inhibit pain. For example, moderate to high doses of DMCM reduce protective paw licking and lifting in response to an intradermal irritant (formalin), elevate paw-lick latency on the hot-plate test, and increase tail-flick latencies to radiant heat (Rodgers and Randall 1987a; Helmsetter et al. 1990; Fanselow et al. 1991; Fanselow and Kim 1992). Blurring the distinction between fear and anxiety, Fanselow and colleagues (1991) have taken the latter findings as evidence that fear inhibits pain.

These divergent effects may be related to other recent observations. Experiments in our laboratory suggest that the unconditioned consequences of shock exposure on pain and learning vary as a function of shock severity: mild to moderate tail-shocks (for example, 3, 0.75-s, 1 mA) enhance pain and learning whereas more severe

shock schedules (3, 25-s, 1 mA; 3, 2-s, 3 mA) attenuate pain and learning (Illich et al. 1995; King et al. 1996; Meagher et al. in review). In a similar fashion, low doses of DMCM could enhance pain and learning whereas high doses have a dampening effect. Indeed, File and Pellow (1988) have shown that a low dose of DMCM (0.1 mg/kg) facilitates passive avoidance learning, whereas a higher dose (0.5 mg/kg) disrupts learning. Another potentially important variable concerns the methodology used to infer a change in pain (Grau et al. 2000). Studies have shown that exposure to the same aversive stimulus (moderate shock) can inhibit selective protective reflexes (for example, tail withdrawal from radiant heat) while at the same time other measures (vocalization to both heat and shock stimuli, and learning) suggest pain is enhanced (Meagher et al. 1990; Illich et al. 1995; King et al. 1996). Interestingly, past studies demonstrating an inhibitory effect of DMCM have relied on measures that do not depend on forebrain systems (for example, protective reflexes) whereas studies demonstrating a facilitatory effect have generally employed measures mediated by learning.

The present study addressed these issues by examining the impact of DMCM over a wide range of doses and with multiple measures of pain reactivity and learning. Our hypothesis was that the outcome obtained would vary depending on both dose and pain measurement technique.

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## General materials and methods

### Subjects

Male Sprague-Dawley rats 100–120 days old (350–400 g), obtained from Harlan (Houston, Tex., USA), were individually housed on a 12/12-h light/dark cycle with ad libitum food and water. The National Research Council Guide for the Care and Use of Laboratory Animals (National Academy Press 1996) was followed throughout both experiments. To minimize the stress of injection, rats were acclimated to being transported and handled for 10 min on the 2 days directly preceding the test day, and were weighed on day 2.

### Apparatus

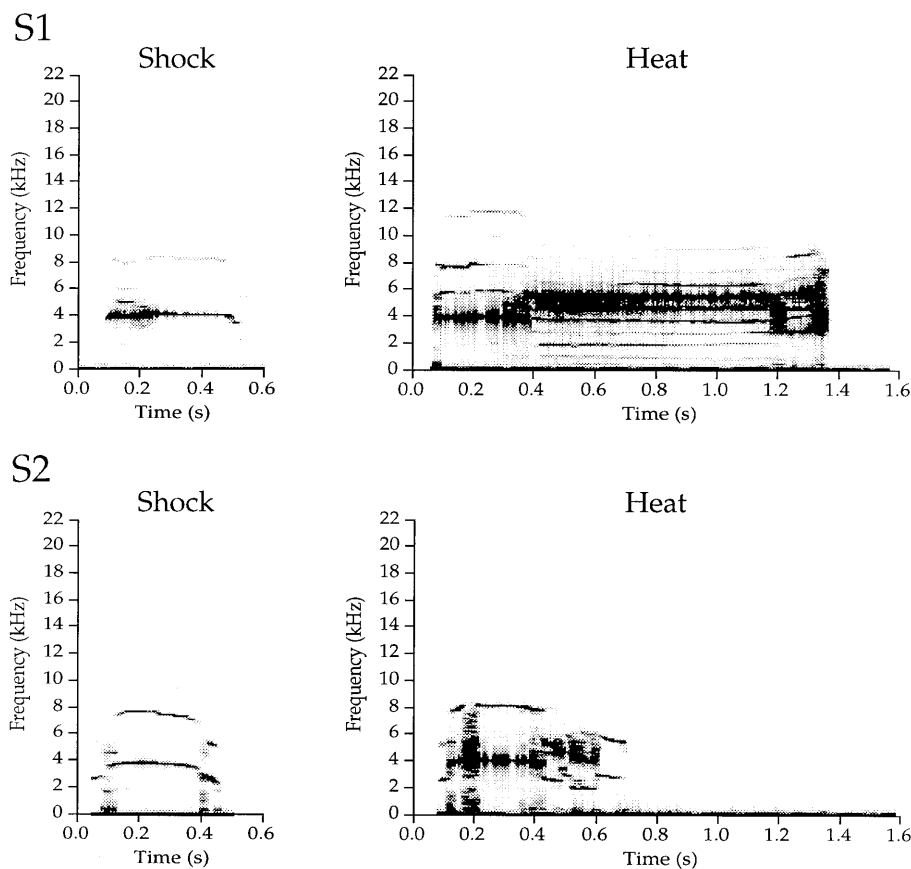
#### *Restraining tubes*

The equipment used to restrain the subjects and test pain reactivity has been described in more detail in a previous publication (King et al. 1996). Briefly, subjects were restrained in opaque Plexiglas tubes (22 cm in length, 6.8 cm in diameter, with a 5.5-cm-wide Plexiglas floor laying 5.3 cm from the top of the tube) with holes drilled in the top of the tube for ventilation. A Plexiglas sheet covered the front of the tube, and a 1.3-cm-wide, 4.3-cm-long strip of Plexiglas extended behind the tube from the middle of the floor. The subjects were able to move freely but not turn around inside of the tube.

#### *Pain reactivity*

A microphone and an automated tail-flick device were used to record the latency to vocalize and tail-flick during the nociceptive

**Fig. 1** Representative sound spectrograms illustrating the vocalization response recorded to shock (*left*) and heat (*right*) for two subjects (*S1* and *S2*). The sounds were recorded at a sampling frequency of 44 kHz using a Power Macintosh 8600 computer. The sound spectrograms were produced with Amadeus II software (v.2.2) (Martin Hairer, Martin.Hairer@math.unige.ch). As reported by Levine and colleagues (1984), subjects exhibited a sonic vocalization during shock that had two primary components. However, the response observed with our shock apparatus was longer (approximately 400 ms) and occurred at a higher frequency. The strongest component was centered around 4 kHz. A weaker component was observed at 8 kHz. These components were also observed in the initial response to a noxious thermal stimulus, but were far stronger. The thermal stimulus also appeared to elicit a secondary component that lasted 0.2–1 s with a strong band between 4 and 6 kHz



stimulus. The microphone was positioned behind a 9.4-mm hole in the front of the tube and is described in greater detail in Illich et al. (1995). The rat's tail rested in a 0.5-cm-deep groove cut into an aluminum block (2.5×6.5×2.5 cm). Located under the groove, a photocell detected a 0.5-cm lateral movement of the rat's tail. A computer monitored the circuit controlled by the microphone as well as the activation of the photocell in the tail-flick device. Once a tail movement and vocalization were recorded, the shock or heat was terminated. To ensure that the tail would remain on the tail-flick device until a vocalization was made, plastic sides (6×6.7 cm) were attached to the aluminum block. Additionally, the blunt end of a wire hook was attached to the tip of the tail with porous tape. It was hooked over an elastic band located 11 cm away from the aluminum block. This held the rat's tail over the aluminum block while still allowing for the tail movement necessary to activate the photocell.

Radiant heat was applied using a condenser lens positioned 8 cm below a 375-W movie light that focused light onto a 2-cm portion of the rat's tail. An AC potentiometer (Leviton, number 6681-W) controlled the heat intensity. Shock was applied to the tail through small electrodes constructed from lightweight fuse clips. A manual shocker (BRS/LVE, model SG-903) produced a continuous variation in shock intensity between 0 and 2 mA. In order to activate the photocell during the shock trials, a small 28-V light (General Instrumental; 1820) was positioned 3.5 cm above the photocell. In accordance with our animal care committee, and to prevent unnecessary pain and suffering, heat was terminated at 8 s and shock at 1.2 mA.

It should be noted that both of the recorded responses (tail movement and vocalization) occur during the presentation of the nociceptive stimulus. Although our equipment can record vocalizations up to 22 kHz, and our stimuli may elicit some ultrasonic vocalizations, the target response was composed of lower frequency components. Sound spectrograms illustrating typical responses to shock and heat are provided in Fig. 1.

#### Conditioned freezing

Pavlovian conditioning was performed using observation chambers (model RTC-021) that were 26 cm wide, 30 cm long, and 38 cm high. The ceiling, front, and rear walls were constructed from clear Plexiglas and the side walls were made of aluminum. Stainless steel rods (0.4 cm in diameter) were spaced 1.5 cm apart to form a grid floor. A 600-V transformer was used in combination with a shock scrambler to administer a constant current, 0.3 mA shock through the grids. The chambers were positioned in test cubicles (40×50×41 cm) and were illuminated by a house light. A fan provided a background noise level of approximately 80 dB. During testing, the cubicle doors were left open. The tubes and observation chambers were situated in separate rooms.

#### Drug

The DMCM was dissolved in 0.01 N HCl and NaOH was used to adjust the pH (Rodgers and Randall 1987a). Subjects were injected with vehicle alone, or 0.015, 0.06, or 0.25 mg/kg DMCM.

### Experiment 1: impact of DMCM on pain reactivity

Experiment 1 examined the impact of DMCM on nociceptive reactivity using two kinds of aversive stimulus (shock and heat) and two dependent variables (vocalization and tail movement). Tail movement to heat (the tail-flick test) is a protective reflex that is spinally mediated and highly sensitive to the antinociceptive effects of both shock and benzodiazepine inverse agonists (Basbaum and Fields 1984; Watkins and Mayer 1986; Rodgers and

Randall 1987a; Fanselow et al. 1991; Morgan et al. 1994). Vocalization represents a supraspinally mediated measure of nociception and has proven sensitive to experimental manipulations thought to enhance pain (Meagher et al. 1990; Illich et al. 1995; King et al. 1996).

It is clear from past studies that high doses of DMCM (>0.25 mg/kg) inhibit nociceptive reactivity (Rodgers and Randall 1987a; Helmstetter et al. 1990; Fanselow et al. 1991; Fanselow and Kim 1992) and, at very high doses (>1.0 mg/kg), motor function (Rodgers and Randall 1987a). Much less is known about the effect of lower doses (<0.25 mg/kg) that theory (King et al. 1996) and data (File and Pellow 1988) suggest may facilitate pain. For these reasons, the present experiment assessed the impact of DMCM on nociceptive reactivity using relatively low drug doses (0, 0.015, 0.06, and 0.25 mg/kg). Sieve (1998) confirmed in our laboratory that DMCM in this dose range reduces both social interaction and the time spent on the open arms of an elevated plus maze, two tests for assessing anxiety-like effects.

Pharmacological studies also suggest that DMCM could have opposing effects at the GABA receptor depending on concentration of the drug. Tietz and colleagues (1999) found DMCM to have a mixed enhancement/inhibition on GABA receptor currents on isolated CA1 pyramidal cells: GABA receptor currents were inhibited at mid-nanomolar concentrations of DMCM, but potentiated in some cells at low nanomolar concentrations.

#### Materials and methods

On the test day, the rats ( $n=32$ ) received subcutaneous injections of the appropriate dose of DMCM, and were then placed in the tubes. After the electrodes and hook were attached to their tails, they were allowed to acclimate while the drug took effect for a time period of 15 min that was based on previous studies (Stephens and Kehr 1985; Rodgers and Randall 1987a; File and Pellow 1988; Fanselow and Kim 1992). In an ABBA order, motor response and vocalization thresholds to heat and shock were measured twice at 6-min intervals. False alarm tests (threshold measurements in the absence of heat and shock) were also conducted before or after the tests (in a counterbalanced order).

#### Results

The impact of DMCM treatment on thermal (lower panels) and shock (upper panels) reactivity is depicted in Fig. 2. There is little indication that low to moderate doses of DMCM facilitate nociceptive reactivity. Instead, DMCM appears to inhibit reactivity in a dose-dependent fashion.

#### False alarms

Mean ( $\pm$  SEM) tail-movement latencies during the shock threshold false alarm trials ranged from 49.25 ( $\pm 6.87$ ) to 59.33 ( $\pm 2.89$ ) s. Mean vocalization response latencies during the false alarm trials ranged from 56.19 ( $\pm 4.25$ ) to

63.10 ( $\pm 0.00$ ) s. None of the subjects responded during the tail-flick false alarms to heat. Mean vocalization latencies during the heat threshold false alarm trials ranged from 7.78 ( $\pm 0.21$ ) to 8.00 ( $\pm 0.00$ ) s. Separate analyses of variance (ANOVAs) confirmed that DMCM did not significantly affect false alarm rate on any of the measures, all  $F_s < 1.13$ ,  $P > 0.05$ .

#### Shock thresholds

Mean tail-movement thresholds to shock across the four doses of DMCM are depicted in the top left panel of Fig. 2. Rats administered the two highest doses of DMCM (0.06–0.25 mg/kg) exhibited higher thresholds. An ANOVA confirmed that the drug manipulation had a significant effect,  $F(3,28)=10.80$ ,  $P < 0.0001$ . To further explore how tail movement thresholds varied as a function of drug dose, a trend analysis was performed. This analysis revealed that the linear and cubic components were significant, both  $F_s > 8.09$ ,  $P < 0.05$ . The cubic trend indicates that there were two significant inflections. Post hoc comparisons using Duncan's multiple range test found that 0.06 and 0.25 mg/kg DMCM significantly increased motor response thresholds to shock compared to the 0.015 mg/kg and vehicle conditions,  $P < 0.05$ . No other differences were significant,  $P > 0.05$ .

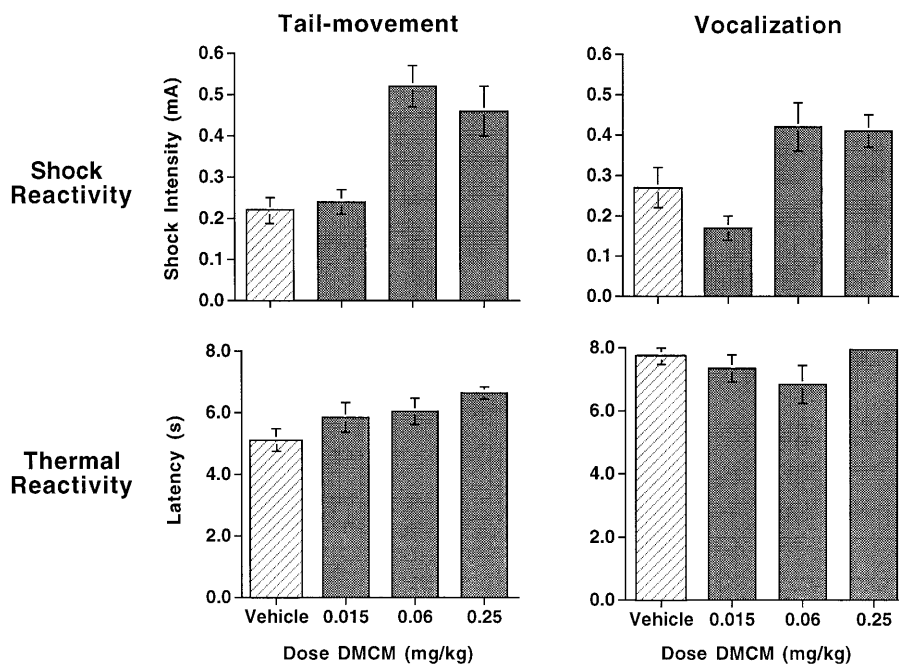
Vocalization thresholds to shock are depicted in the top right panel of Fig. 2. As usual, higher shock intensities were needed to elicit a vocalization response but this difference was somewhat smaller than that observed in prior studies (cf. Illich et al. 1995). More importantly, the two highest doses raised shock thresholds. An ANOVA showed that the drug manipulation had a significant impact on vocalization thresholds,  $F(3,28)=5.58$ ,  $P < 0.005$ . A trend analysis revealed significant linear and cubic components, both  $F_s > 6.90$ ,  $P < 0.05$ . The quadratic component was not significant,  $F(1,28)=0.88$ ,  $P > 0.05$ . A Duncan's multiple range analysis found that 0.06 and 0.25 mg/kg DMCM increased vocalization thresholds to shock compared to the 0.015-mg/kg dose,  $P < 0.05$ . No other differences were significant,  $P > 0.05$ .

#### Heat thresholds

Tail-flick latencies to heat are presented in the bottom left panel of Fig. 2. Here too DMCM produced a systematic increase in nociceptive thresholds. Although the overall ANOVA did not reach statistical significance,  $F(3,28)=2.35$ ,  $P > 0.05$ , trend analysis revealed a significant linear component  $F(1,28)=6.78$ ,  $P < 0.05$ . The quadratic and cubic components were not significant, both  $F_s < 1.0$ ,  $P > 0.05$ . Post hoc comparisons showed that the 0.25-mg/kg group differed from the vehicle controls,  $P < 0.05$ . No other differences were significant,  $P > 0.05$ .

Vocalization latencies to heat are depicted in the bottom right panel of Fig. 2. Although it appears that intermediate doses lowered vocalization thresholds, this effect

**Fig. 2** Tail-movement (left panels) and vocalization (right panels) latencies to shock (top panels) and thermal (bottom panels) stimuli following administration of 0, 0.015, 0.06, or 0.25 mg/kg DMCM are depicted in mA and seconds, respectively



did not approach statistical significance,  $F(3,28)=1.19$ ,  $P>0.05$ . Trend analysis also failed to reveal any significant differences, all  $F_s<1.0$ ,  $P>0.05$ .

## Discussion

A high dose of DMCM (0.25 mg/kg) induced hypoalgesia on three of the four measures of nociceptive reactivity, providing converging evidence that the drug inhibits pain. The one exception was vocalization to thermal stimulation. However, the long latencies observed in the vehicle controls produced a ceiling effect that made it difficult to resolve a further increase on this dependent variable. Taken together, these data provide converging evidence that DMCM does not lower nociceptive thresholds.

Our hypothesis was that lower doses of DMCM might induce hyperalgesia and that this effect could depend on how pain reactivity was assessed. We were particularly interested in the impact of DMCM on vocalization thresholds, for past studies suggest that this target response should be especially sensitive to hyperalgesia (Illich et al. 1995; King et al. 1996). Using this measure that favors hyperalgesia, but makes it difficult to resolve antinociception, there was a hint of hyperalgesia at the 0.015 (vocalization to shock)- and 0.06 (vocalization to heat)-mg/kg doses. However, these effects did not reach statistical significance. Even if these conditions are directly compared to the vehicle controls, statistical significance was not obtained, both  $F_s<2.28$ ,  $P>0.05$ . We also independently assessed the impact of these doses on an additional 12 subjects and again failed to observe a significant hyperalgesic response, even when the data were pooled across replications, all  $F_s<1.46$ ,  $P>0.05$ . Clearly, if DMCM induces hyperalgesia, this effect is masked by the drug's inhibitory effect on behavioral reactivity.

## Experiment 2: impact of DMCM on learning

It is possible that the inhibitory effect of DMCM on nociceptive reactivity reflects a motor effect rather than a reduction in pain perception. The present experiment addresses this issue using an alternative test that relies on Pavlovian conditioning. The logic is that a manipulation that influences pain should alter the capacity of an aversive event to support fear conditioning. We test this by presenting a weak (0.3 mA) grid shock in a distinctive context in the presence of DMCM. Plasma benzodiazepine receptor binding activity of DMCM rapidly declines, after peaking at 15–30 min following administration (Boer et al. 1991). Thus, the next day subjects can be re-exposed to the context alone (after the effects of DMCM in the animal's system have diminished) and the amount of conditioned freezing is recorded. We have previously shown that manipulations thought to induce hyperalgesia enhance the acquisition of conditioned freezing while manipulations that induce hypoalgesia undermine learning (King et al. 1996; Meagher et al. in review). For present purposes, by measuring the amount of freezing on day 2 this test has a unique advantage; it allows us to examine the impact of DMCM on pain after the drug has cleared the system, effectively reducing the contaminating influence of the drug's effect on motor function. By eliminating the motoric effects, we may unveil a drug-induced enhancement of pain.

Alternatively, DMCM could block learning in a dose-dependent fashion. Indeed, there is considerable evidence that unsignaled shocks condition fear to the training context and that this conditioned state undermines learning new cues predict shock, an example of the US pre-exposure effect (Randich and LoLordo 1979). However, this effect is generally characterized in terms of specific stimulus representations/expectations rather than

diffuse motivational states (see, for example, Rescorla and Wagner 1972; Wagner 1981). If DMCM blocks learning it would suggest that the induction of a negative affective state is sufficient to prevent learning. Although such an outcome could be derived within a model that separates sensory and emotional processing (Wagner and Brandon 1989), it is most naturally derived from the PDR theory outlined by Bolles and Fanselow (1980).

#### Materials and methods

Rats ( $n=48$ ) were injected with the appropriate dose of DMCM and placed in the restraining tubes for 15 min while the drug took effect. Subjects were then transferred to the conditioning chamber where their baseline freezing behavior was videotaped for 3 min. Half of the rats in each drug condition then received a mild shock (0.5 s, 0.3 mA) while the remaining rats received no shock. Their behavior was recorded for an additional 2 min after shock treatment. Twenty-four hours later, the rats were returned to the same chamber and their freezing behavior was recorded for 8 min.

Behavior during the training and testing trials was scored by an observer who was blind to the rat's drug treatment. The activity level of the rat was assessed at 3-s intervals and was scored as freezing or activity. Freezing was defined as the absence of all visible movement of the body, except for movement necessary for respiration. All other behaviors were scored as activity. Further details can be found in Grau (1984) and Fanselow (1984).

#### Results

The mean percentage of time spent freezing, as well as the mean incidence of freezing, for all drug conditions during training (day 1) and testing (day 2) is presented in Fig. 3. We found that the highest dose of DMCM (0.25 mg/kg) increased freezing prior to shock treatment and blocked learning. Lower doses had no effect.

##### *Baseline freezing levels*

The percent of freezing prior to shock treatment (baseline) is presented in the leftmost panels of Fig. 3. The highest dose of DMCM induced freezing. An ANOVA revealed that DMCM treatment had a significant impact on the percent of freezing,  $F(3,40)=11.62$ ,  $P<0.0001$ . No other differences were significant,  $F<1.0$ ,  $P>0.05$ . A Duncan's multiple range analysis showed that the 0.25 mg/kg DMCM group froze significantly more than all other groups,  $P<0.05$ . No other differences were found,  $P>0.05$ .

##### *Day 1 freezing levels after shock*

The percent of freezing observed during the 2-min period following shock treatment is depicted to the right of the baseline scores. Shocked rats generally froze more and this effect was blocked by the highest dose of DMCM (0.25 mg/kg). ANOVAs detected significant effects of dose and shock, and a Shock  $\times$  Dose interaction, all  $F_s>3.67$ ,  $P<0.05$ . Duncan's multiple range analyses

found significant differences between the shocked group and unshocked groups for the vehicle, 0.015, and 0.06 mg/kg DMCM conditions,  $P<0.05$ . However, the unshocked and shocked groups for the 0.25 mg/kg DMCM condition were only significantly different from the unshocked groups of the other doses, not from each other,  $P<0.05$ . No other differences were significant,  $P>0.05$ .

##### *Day 2 freezing levels*

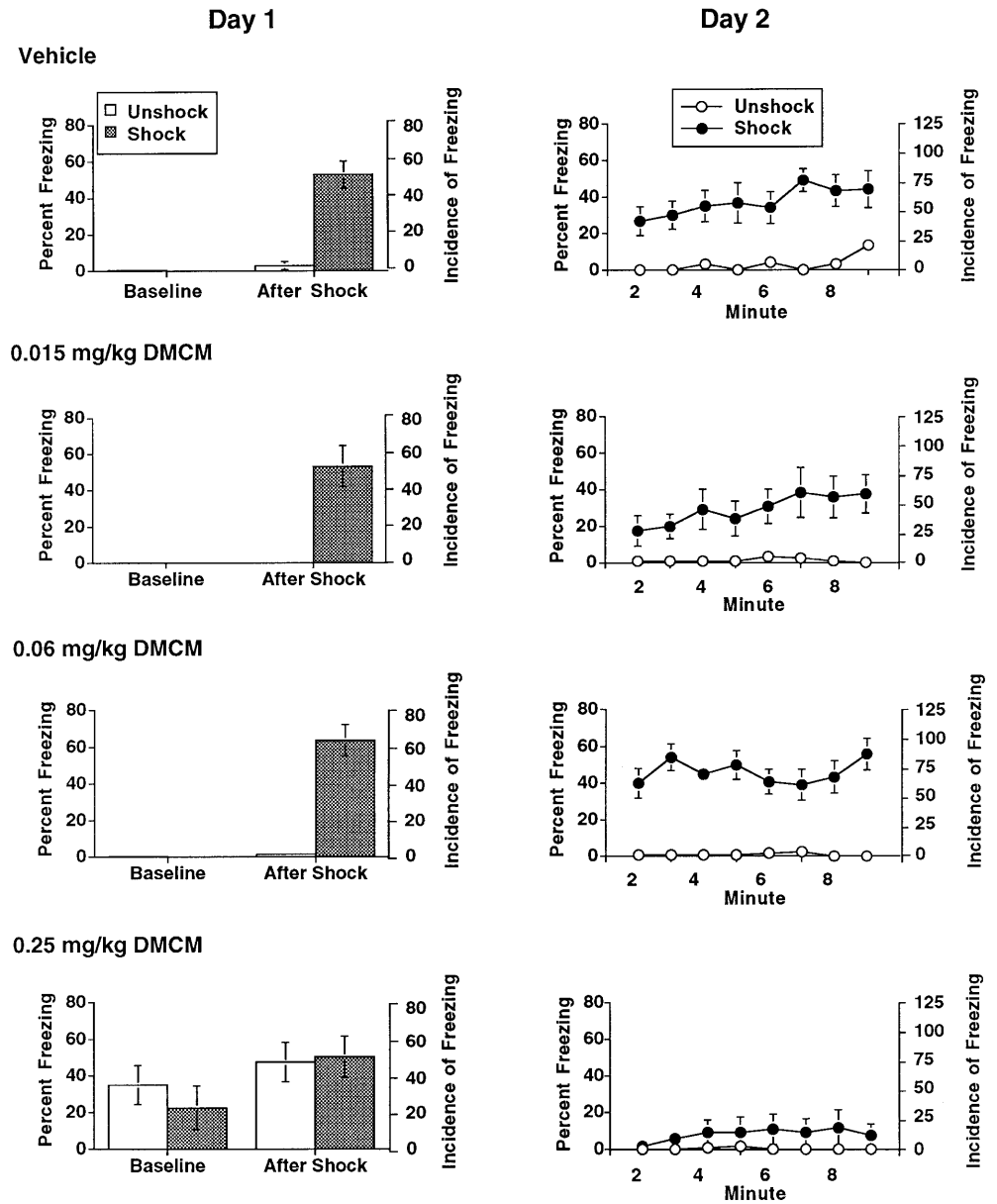
The levels of freezing observed over time (min) are presented in the rightmost panels of Fig. 3. Previously shocked rats froze more than unshocked rats and this effect was blocked by the highest dose of DMCM (0.25 mg/kg). ANOVAs revealed significant effects of shock, dose, and minute of testing as well as a Shock  $\times$  Dose interaction, all  $F_s>2.68$ ,  $P<0.05$ . Duncan's multiple range analyses showed that shocked rats given 0, 0.015, or 0.06 mg/kg DMCM froze significantly more than all other groups,  $P<0.05$ . Shocked rats given 0.06 mg/kg DMCM also froze significantly more than those given 0.015 mg/kg,  $P<0.05$ . No other differences were significant,  $P>0.05$ .

#### Discussion

As previously reported, saline-treated shocked rats exhibited freezing, both immediately after shock and the next day when they were re-exposed to the shock chambers. A low dose of DMCM (0.015–0.06 mg/kg) did not have a significant effect on the amount of freezing observed either before or after shock treatment. In contrast, the highest dose of DMCM (0.25 mg/kg) reduced activity prior to shock treatment and blocked the acquisition of conditioned fear to the chamber, as evidenced by the reduced freezing observed on day 1 and day 2. To our knowledge, this is the first demonstration that pharmacologically inducing a negative affective state can block learning. Given the results of experiment 1, we assume that this effect is attributable to a drug-induced hypoalgesia that reduced the reinforcing capacity of the US.

We suggested above that our conditioning procedure provides a way to disentangle the drug's impact on motor function from its effect on pain. Indeed, there was reason to believe that the drug enhances pain, but inhibits motor function (Stephens and Kehr 1985; File and Pellow 1988). If so, DMCM-treated shocked rats should have exhibited enhanced conditioned fear when they were re-exposed to the shock chamber 24 h later. There was a hint that an intermediate dose (0.06 mg/kg) has this effect, but our post hoc comparisons did not reveal a statistically significant difference. To further explore the impact of the 0.06-mg/kg dose on conditioned freezing, we performed an additional set of analyses to directly compare this group to the vehicle group. Again, neither the main effect of drug treatment, its interaction with shock treatment, or their interaction with minute of testing approached statistical significance, all  $F_s<1.46$ ,  $P>0.05$ .

**Fig. 3** Mean conditioned freezing levels on day 1 (*left panels*) for the baseline period (*left*) and following a 0.5-s, 0.3-mA grid shocks or an equivalent period of time (*right*) for shocked (*filled bars*) and unshocked (*open bars*) rats administered 0, 0.015, 0.06, and 0.25 mg/kg DMCM (*top to bottom panels*). Conditioned freezing on day 2 (*right panels*) is presented over time for shocked (*filled circles*) and unshocked (*open circles*) rats in each drug condition



## General discussion

Prior studies have shown that a high dose of DMCM (>0.5 mg/kg) inhibits nociceptive protective reflexes (for example, tail and paw withdrawal) and general activity (Rodgers and Randall 1987a; Helmstetter et al. 1990; Fanselow et al. 1991; Fanselow and Kim 1992). There have been relatively few studies examining the impact of low to moderate doses of DMCM (<0.25 mg/kg) and these have yielded mixed results (Rodgers and Randall 1987a; File and Pellow 1988). Because the impact of a noxious stimulus varies as a function of both stimulus severity and test type (Illich et al. 1995; King et al. 1996; Grau et al. 2000), we hypothesized that the effect of DMCM would vary as a function of dose and pain measurement technique. Specifically, we expected that a low dose may inhibit the protective reflex of tail withdrawal

but enhance vocalization and learning, while a high dose has a general inhibitory effect. This pattern was also anticipated by our theoretical claim that anxiety induces hyperalgesia and that more intense negative affective states (fear) yield hypoalgesia.

The assumptions underlying these experiments were simple and clear; if anxiety enhances pain, then administration of an anxiogenic should have a facilitory effect on nociceptive reactivity and learning. Given our theoretical predictions (King et al. 1996; Rhudy and Meagher 2000), we designed the experiments in favor of nociceptive facilitation, using doses that should minimize motoric inhibition and tests (vocalization and learning) that have proven highly sensitive to hyperalgesia (Illich et al. 1995; King et al. 1996). Even under these conditions we obtained evidence for pain inhibition. Instead of a biphasic effect, DMCM produced a dose-dependent inhibition

**Table 1** Modes of defense (modified from Fanselow 1994). *dIPAG* Dorsal and lateral region of the periaqueductal gray, *vPAG* ventral periaqueductal gray

Defensive mode	Psychological state	Eliciting stimuli	Neural structures	Behavioral/psychological consequences
Pre-encounter	Anxiety	Unexpected aversive stimuli Risk of predation	dIPAG Central amygdala BNST	Hyperalgesia Enhanced startle Meal pattern reorganization Stretched approach
Post-encounter	Fear	Innate and learned signals of danger	vPAG Central amygdala Basolateral amygdala	Opioid hypoalgesia Enhanced startle Freezing
Circa-strike	Panic	Direct, or inevitable, contact with a noxious or life-threatening stimulus	dIPAG Superior colliculus	Non-opioid hypoalgesia Diminished startle Diminished conditioned freezing Fight/flight

of both protective reflexes and vocalization (experiment 1). Experiment 2 used a remote test based on Pavlovian conditioning. If DMCM enhanced the aversive quality of shock, it should have increased its capacity to support learning. However, the opposite outcome was obtained, providing converging evidence that the drug inhibits pain.

In many regards, these observations parallel those obtained using the acoustic startle paradigm. Evidence suggests that the induction of anxiety and/or fear facilitates the acoustic startle response in animals and humans (Davis 1989; Davis et al. 1997; Walker and Davis 1997). Given this, administration of an anxiogenic should enhance startle. Yet, Fanselow et al. (1991) showed that DMCM inhibits acoustic startle. Again, the implications seem clear: the state induced by DMCM does not have a facilitatory effect.

At a theoretical level, there are three potential interpretations of these problematic results. One alternative is that DMCM induces anxiety, which has a facilitatory effect, but this effect is masked by a secondary effect (for example, motor inhibition). The second possibility is that moderate doses of DMCM induce anxiety (the drug is widely considered an anxiogenic), but anxiety does not facilitate pain or startle. Finally, anxiety may facilitate pain and startle, but perhaps DMCM does not induce a state of anxiety. In the sections that follow, we consider each alternative in turn.

First, it is possible that DMCM-induced hyperalgesia was masked by a secondary inhibitory effect. Though this alternative cannot be completely rejected, pain inhibitory effects were observed at relatively low doses that had little (0.25 mg/kg) or no (0.06 mg/kg) effect on general activity (experiment 1). More importantly, evidence of pain inhibition was obtained using a remote test (experiment 2), when the drug was no longer present. It would appear that the most parsimonious conclusion is that the state elicited by systemic DMCM treatment does not have a facilitatory effect.

Interpretation of these data then depends on whether DMCM is an anxiogenic. If DMCM is an anxiogenic, we must reject the claim that anxiety facilitates pain (alter-

native 2). Likewise, the notion anxiety enhances startle would be called into question. Such a conclusion would force us to reconsider how shock effects pain and startle, for we could no longer attribute the unconditioned facilitation to the induction of anxiety. How else could we characterize the underlying state? Perhaps the crucial component actually reflects a precursor to anxiety, a kind of unexplained arousal (Barlow et al. 1996). In a similar vein, Davis (1989) has suggested that shock-induced sensitization of acoustic startle provides a “read-out” of amygdala activation that could be linked to a critical period for memory consolidation. Both characterizations bear a relation to the notion that unexpected events elicit a form of surprise that fosters learning. From this perspective, a variety of events could induce a state of unexplained arousal (for example, brief shock, omission of an expected stimulus event) and this state could generally enhance stimulus processing (Gallagher and Holland 1994). This would increase the hedonic impact of stimuli that naturally, or as a consequence of learning, elicit pain or negative affect. Paradoxically, it could also enhance the affective impact of pleasant stimuli (for example, drug reinforcement, sexually related stimulation). We are currently exploring these possibilities.

The last possibility is that anxiety induces hyperalgesia, but DMCM does not induce anxiety. Given that DMCM is generally characterized as an anxiogenic, it would appear that we could reject this alternative without further consideration. Yet, there are reasons to suspect that the drug induces a state that differs in important ways from what we normally think of as anxiety. To understand how this could be, we need to unpack the presumed relationship between negative affect and pain. The theoretical framework that has been implicitly assumed was derived from Fanselow’s (1994) characterization of defensive modes and is illustrated in Table 1. Following Rodgers and colleagues (1987b, 1995) and Fanselow (1994), we assume that these distinct modes evolved to subserve antipredator defense, that the mode engaged depends on the nature of the eliciting stimulus, and that distinct pain modulatory effects can be linked to different regions of the periaqueductal gray (PAG). The

ventral PAG (vPAG) is important for freezing and opioid hypoalgesia (postencounter defense), and is engaged by the expectation of aversive stimuli such as shock (Fanselow 1994; De Oca et al. 1998). Neural systems in the dorsal and lateral region of the PAG (dIPAG), are engaged under even more dire circumstances (direct contact with a severe aversive stimulus) and have been linked to fight, flight, reactive immobility, and non-opioid hypoalgesia (circa-strike defense; Depaulis et al. 1992; Fanselow 1994). At a psychological level, freezing behavior and opioid hypoalgesia have been linked to the induction of fear (Bolles and Fanselow 1980; Fanselow 1994). Direct contact with a noxious event appears to induce a more intense negative affective state that others have characterized as a state of panic (Graeff et al. 1993; Jenck et al. 1995). Our recent work has focused on a third mode of defense that is elicited under low to moderate levels of aversive stimulation (Meagher et al. in review). Like the circa-strike mode, it depends on the dIPAG (McLemore et al. 1999) and may occur as an unconditioned response to aversive stimulation (Illich et al. 1995). However, it has the opposite effect on pain and learning, sensitizing both, an outcome indicative of hyperalgesia. This sensitization could help prepare the organism for danger, yielding a form of pre-encounter defense. Psychologically, we have suggested that this state could be tied to a state of anxiety (King et al. 1996; Crown et al. 2000).

Prior parametric studies have shown that these three modes of defense can be sequentially engaged by increasing shock severity (duration, intensity, and/or density; Fanselow and Lester 1988; Meagher et al. in review), and rely on distinct neural systems (Meagher et al. 1993; Fanselow 1994; Grau et al. 1996; Crown et al. 2000). Following Fanselow (1994), we assumed that these distinct modes of defense can likewise be sequentially engaged by different levels of negative affect. If so, DMCM should elicit behaviors indicative of anxiety, fear, and panic, in a dose-dependent fashion. From this perspective, a moderate dose of DMCM would engage the vPAG-mediated postencounter mode associated with fear and elicit both freezing and opioid hypoalgesia. Supporting this, the lowest effective dose that produced hypoalgesia on the formalin test also reduced activity (Fanselow et al. 1991). However, the hypoalgesia observed was insensitive to an opioid antagonist, an observation that appears to implicate the dIPAG, not the vPAG. Likewise, the drug-induced inactivity seemed different from that produced by a fear-eliciting stimulus, for the latter is associated with enhanced startle. Yet DMCM inhibited acoustic startle. In this regard, the drug-induced state seemed more akin to tonic immobility than fear-induced freezing.

These observations suggest that systemic DMCM, at behaviorally relevant doses, engages the circa-strike mode of defense. This is consistent with an idea put forth by Rodgers and colleagues (1987b, 1995), who suggested that the non-opioid hypoalgesia observed during fight/flight may be attributable to the release of an en-

dogenous benzodiazepine ligand that has inverse agonist-like properties. Psychologically, the circa-strike mode of defense may reflect a form of panic (Dorow et al. 1983). Indeed, humans given DMCM often used this descriptor and the behavioral signs (for example, profuse sweating, mutism) seem in line with a panic attack. Further, if DMCM engages the circa-strike mode of defense, the drug should inhibit both learning and acoustic startle. As we have seen, both are inhibited. But what of the drug's effect on social interaction and the elevated plus maze? This could reflect either the drug's inhibitory effect on motor behavior or the induction of panic. The implication is that DMCM may induce an intense negative affective state, but this state may be more like panic than anxiety. If this is true, then it is possible that anxiety does enhance pain and startle as hypothesized. DMCM could have a paradoxical effect, not because our hypothesis is wrong, but rather because the drug is not truly an anxiogenic.

Why would DMCM differentially elicit a panic-like state, instead of dose dependently producing a gradient of negative affect corresponding to anxiety, fear, and panic? It has been suggested that systemic DMCM influences affect and defensive behavior through GABAergic sites within the amygdala, PAG, and dorsal raphe (Fanselow and Kim 1992; Maier et al. 1995). Perhaps these sites interact in a synergistic fashion, yielding a kind of multiplicative interaction that amplifies the drug's psychological and behavioral consequences. Concurrently engaging these multiple GABAergic mechanisms could yield a panic-like state rather than anxiety. Furthermore, the activation of GABAergic systems in both the vPAG and dIPAG could suppress behavior in multiple ways, yielding a state more like tonic immobility than freezing.

It is important to recognize that this alternative characterization of the drug's effect does not pose a theoretical challenge to PDR theory, for Fanselow (1986, 1994) has extended the original model to encompass a state of intense fear. From this perspective, intense fear undermines learning because it induces a non-opioid hypoalgesia. Further, the fact that DMCM may engage just one state does not imply that other kinds of events (for example, the expectation of pain) engage a single mode of defense. Nor does this proposal imply that panic does not affect anxiety. Indeed, there is evidence that a high dose of DMCM induces a long-term sensitization that predisposes subjects to becoming anxious and/or fearful (Drugan et al. 1985; Maier et al. 1995). Our only qualification to these results is that the inducing event may be better described as a state of panic than prolonged anxiety.

In summary, the present study showed that DMCM induces hypoalgesia on a wide range of assays and under conditions designed to minimize the confounding effects of the drug on motor reactivity. Further, we showed that pharmacologically inducing a negative affective state can block Pavlovian fear conditioning. It is suggested that DMCM induces a state of panic that inhibits pain.

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