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Evaluation of a post-operative pain-like state on motivated behavior in rats: Effects of plantar incision on progressive-ratio food-maintained responding

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Abstract

There has been recent interest in characterizing the effects of pain-like states on motivated behaviors in order to quantify how pain modulates goal-directed behavior and the persistence of that behavior. The current set of experiments assessed the effects of an incisional post-operative pain manipulation on food-maintained responding under a progressive-ratio (PR) operant schedule. Independent variables included injury state (plantar incision or anesthesia control) and reinforcer type (grain pellet or sugar pellet); dependent variables were tactile sensory thresholds and response breakpoint. Once responding stabilized on the PR schedule, separate groups of rats received a single ventral hind paw incision or anesthesia (control condition). Incision significantly reduced breakpoints in rats responding for grain, but not sugar. In rats responding for sugar, tactile hypersensitivity recovered within 24 hrs, indicating a faster recovery of incision-induced tactile hypersensitivity compared to rats responding for grain, which demonstrated recovery at PD2. The NSAID analgesic, diclofenac (5.6 mg/kg) completely restored incision-depressed PR operant responding and tactile sensitivity at 3 hr following incision. The PR schedule differentiated between sucrose and grain, suggesting that relative reinforcing efficacy may be an important determinant in detecting pain-induced changes in motivated behavior.

Keywords

progressive ratio schedules; operant conditioning; preclinical pain assay; nociception; post-operative incisional pain; pain-depressed behavior

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INTRODUCTION

There has been a concerted effort to develop preclinical assays of nociception that can serve as valid indicators for quantifying pain-like states, and provide suitable predictive platforms for therapeutics development for both veterinary and human populations [Roughan & Flecknell, 2000, 2001, 2003; Martin et al., 2004, 2005; Stevenson et al., 2006; Do Carmo et al., 2009; King et al., 2009; Sotocinal et al., 2011; Freitas et al., 2015; Negus et al., 2015]. One of the approaches from our laboratories has been to characterize the effects of pain on normally performed, unconditioned behaviors in rodents such as free-access feeding, open-field locomotor activity, and wheel running [Stevenson et al., 2006, 2009, 2011]. The resulting pain-related depression of behavior is consistent with the behavioral change seen in veterinary and human clinics, and restoration of pain-depressed behavior is one of the goals of effective antinociception and analgesia. A distinct challenge in utilizing unconditioned activity patterns as dependent variables is the high degree of within-subject and between-subject baseline variability [Stevenson et al., 2011]. This kind of natural behavioral variation is certainly seen in pain clinics [IOM, 2011], but a large degree of variance can make it difficult to detect the influence of independent variables on target behaviors.

A recent trajectory in preclinical pain studies has been the innovative utilization and refinement of operant procedures to assess the effects of pain-like states on conditioned behavior [Martin et al., 2004, 2005; Vierk et al., 2004; Neubert et al., 2005; Morgan et al., 2008; Freitas et al., 2015]. In an operant conditioning experiment, the subject performs a response (e.g., a lever press) in the presence of one or more stimuli (e.g., a stimulus light located above the lever), to receive a reinforcer (e.g., a food pellet). Although operant procedures can require extensive training, they may hold promise for determining pain-induced changes to motivated behavior, and provide a daily index for an affective-like dimension of pain. An additional feature of these procedures is that they provide exquisitely stable baselines of behavior [Ferster & Skinner, 1957], which have been shown to be highly sensitive to environmental manipulations including nociception [Martin et al., 2004; 2005].

The purpose of the present study was to evaluate the hypothesis that an incisional pain manipulation can suppress motivated behavior in rats. A secondary goal was to determine if this pain-related depression of behavior could be restored with a standard analgesic. Toward that end, the effect of plantar paw incision was determined on progressive-ratio operant performance in rats trained to respond for either grain or sugar pellet reinforcers. A progressive-ratio (PR) operant schedule is one in which the number of lever presses required for each successively earned reinforcer progressively increases according to an arithmetic or logarithmic function [Hodos, 1961]. Although there have been several reports using simple schedules of reinforcement in the pain literature, there has been (as far as the authors know) only one recently published paper utilizing a PR schedule of food-maintained reinforcement [Schwartz et al., 2014]. In that report the pain-suppressed behavior was not restored with a standard analgesic, calling into question the inference that pain was the primary causal factor for decreases in responding. However, the same authors include neurobiological data that is interpreted to suggest the possibility that PR schedules may be useful for quantifying motivational aspects of injury-modified behavior. The PR schedule has been widely used in drug self-administration studies to quantify and compare relative reinforcing efficacy of

drugs of abuse [Griffiths et al., 1975; Winger & Woods, 1985; Woolverton, 1995], and to assess the effects of neurological, pharmacological and environmental manipulations on schedule-controlled responding for food [LeSage et al., 2004; Bezzina et al., 2008; Fletcher et al., 2010; Overduin et al., 2012; Blaisdell et al., 2014]. The primary dependent variable in PR studies is the breakpoint, defined as the final ratio completed. PR schedules for food-maintained responding are interpreted to measure the reinforcing efficacy or motivational strength of food [Hodos & Kalman, 1963].

The PR schedule was chosen as a behavioral endpoint in the present set of experiments because it provides a way to model work effort and may represent a simple form of the complex psychological construct of motivated behavior [Myers, 2013]. An incisional pain manipulation was chosen because it closely mimics the clinically relevant post-operative pain state, and developing more effective treatments of post-operative pain for veterinary and human populations has been an ongoing challenge in the pain field. The NSAID, diclofenac, was chosen as the reference analgesic because it has been shown to be effective in treating both human and veterinary inflammatory pain conditions, and NSAIDs, in general, have been verified in preclinical studies [Whiteside et al., 2008; Spofford et al., 2009; Xie et al., 2014].

MATERIALS AND METHODS

Subjects

Adult male Sprague Dawley rats (200–250 g at the start of the experiment; Harlan, Indianapolis, IN) were used for all studies. Rats were housed singly in standard Plexiglas containers with water available ad libitum. Rats were maintained at 85% free-feeding weight for the duration of the experiment. Animals were maintained in a temperature and humidity controlled colony on a 12-h light/dark cycle (lights on at 8:00am). All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health. The University of New England Institutional Animal Care and Use Committee (IACUC) approved all protocols involving animals.

Operant Conditioning Experiments

Apparatus—All studies were conducted in operant conditioning chambers (Med Associates, Model MED-008-B1) placed within sound-attenuating cubicles equipped with a house light and exhaust fan. Each chamber contained two response levers situated on the front wall of the chamber. A shallow steel cup situated between the two levers, and just above the floor, contained a reservoir consumption of food pellets. A pellet dispenser delivered 45 mg sugar or grain-based food pellets (see detailed methods below). Stimulus lights were situated above each response lever and were programmed to signal the availability of food.

Food training—Lever pressing was initially shaped during daily training sessions (30 min day 1; 15 min days 2–8). Food training involved reinforcement of successive approximations of lever-press behavior with delivery of a food pellet. Once shaping was complete, 45 mg food pellets (Noyes brand) were available under a Fixed Ratio (FR) 1

schedule of reinforcement for a 30 min session. In a fixed ratio schedule the subject emits a fixed number of responses for each reinforcer. Thus, an FR1 schedule indicates that 1 response is required to obtain each reinforcer. Illumination of the stimulus light above the active lever served as a discriminative stimulus that the response-reinforcer contingency was in place. Responding on the other inactive lever was counted but had no programmed consequences (animals were counterbalanced such that the left lever was active for half the rats and the right lever was active for the other half). A maximum of 50 food reinforcers was available during each daily training session. Once responding stabilized under the FR1 schedule (three consecutive days in which response rates varied by no more than 20% and at least 70% of responses emitted on the active lever), session length was increased to 3hr for three consecutive days, to determine the maximum number of pellets that could be earned in a 3hr session. Following the 3 hr sessions, session length was set back to 30min and once responding was stable at 30 min, the FR requirement was gradually raised to FR5, such that 5 responses were required to obtain each reinforcer. After responding stabilized under the FR5 schedule, using the same criteria as above, half of the rats were switched to sugar pellets as their reinforcer (the other half remaining on grain pellets), and each reinforcer remained for the duration of the experiments.

Progressive ratio schedule—Once responding was stable under FR5 with grain or sugar pellets, rats were assigned to a progressive ratio schedule that contained a logarithmic step sequence [Richardson and Roberts, 1996] according to the following formula $[(5e^{(\text{reinforcer number} \times j)} - 5)]$, where $j=0.17$, yielding a sequence of 1, 2, 3, 5, 7, 9, 11, 14, 18, 22, 27, 33, 41, 49... The PR schedule was programmed with a limited hold (LH) of 15 min, such that if they did not earn a reinforcer within 15 min, the session timed out. This schedule was chosen based on pilot studies in our laboratory [Stevenson et al., unpublished data] that characterized several different PR schedules with arithmetic or logarithmic sequences, and different LH values. The current PR schedule was selected because it contained (1) relatively high breakpoints, (2) low intra-animal variability in breakpoints across 5 consecutive days (defined as 5 consecutive days in which breakpoints varied by no more than 15%), and (3) session lengths shorter than 30 min (to facilitate analgesic drug testing and increase throughput). Once responding stabilized on the PR schedule, rats were divided into four groups to characterize the effects of incision on grain-maintained or sugar-maintained responding. The groups were: incision + grain, anesthesia control + grain, incision + sugar, anesthesia control + sugar. For the incision manipulation, operant responding was measured 3hr, 1, 2, 3, and 7 days post-incision, and for an additional two weeks. Operant conditioning experiments were run between 10am and 3pm, Monday thru Friday.

Incisional pain manipulation

Animals were anesthetized with isoflurane (2–3%) vaporized via a nose cone. The plantar aspect of the left hind paw was prepared in a sterile manner with 5% povidone-iodine solution and 70% alcohol. A 1-cm long incision, starting 0.5cm from the heel and extending toward the toes, was made with a number 10 blade, through skin and fascia of the plantar aspect of the left hind paw including the underlying muscle [Brennan et al., 1996]. The plantaris muscle was then elevated and longitudinally incised with a number 11 blade,

leaving the muscle origin and insertion intact. Hemostasis was reached using a gauze pad and gentle pressure to the wound site. The skin was then closed with two mattress sutures of 5-0 nylon. The paw was cleaned with saline and a topical antibiotic was applied to prevent infection. At the end of the surgery, anesthesia was stopped and animals were allowed to recover before being returned to their home cages. The incision manipulation was performed as a within-group design, such that rats that received anesthesia only, subsequently received anesthesia + incision four weeks later, and vice versa. Importantly, PR performance of animals that received anesthesia only did not differ between the groups that previously received incision and those that did not.

Von Frey Assay

Tactile allodynia was assessed using von Frey monofilaments. All von Frey experiments were conducted 1 h following operant experiments. Once operant sessions were complete, rats were removed from their operant chamber and placed in a Plexiglas cage with a wire mesh bottom. The rats were allowed to acclimate for 15 min (or until exploratory and grooming behavior declined to a level compatible with behavioral testing). Von Frey monofilaments were applied to the mid-plantar left hind paw (ipsilateral side of incision) with a series of monofilaments that ranged in stiffness from 0.4 to 15 g (0.4, 0.6, 1, 2, 4, 6, 8, 15 g). Filaments were applied once for 5 s with inter-stimulus intervals of 1 min. Rats were tested using the up-down method [Chaplan et al., 1994]. Briefly, rats were tested beginning with the 2 g monofilament. If the rat did not emit a positive response (defined as a rapid withdrawal and/or licking of left hind paw), the next highest filament in the sequence was tested until the rat showed a positive response or reached maximum threshold. If the rat did respond to the 2 g monofilament, the next lowest filament was used until the rat stopped emitting a positive response. Baseline measures were recorded several days prior to pain manipulations. For the incision experiments, rats were tested with von Frey monofilaments 6 hr, 1, 2, 3, and 7 days post-incision. Testing was completed between 11am and 4pm.

Drug

The nonsteroidal anti-inflammatory drug (NSAID), diclofenac (Sigma) was dissolved in sterile saline and administered i.p. 30 minutes prior to operant experiments. The 5.6 mg/kg dose was chosen based on pilot data in our lab, and literature on diclofenac efficacy and potency against a range of nociceptive conditions [Euchenhof et al., 1998; Moller et al., 2012; Yoshimi et al., 2010; Zhang et al., 2012].

Data Analysis

The dependent variable for operant experiments was breakpoint (defined as the final ratio completed). Breakpoint was transformed to 100% control, with control responding representing 3 consecutive days of PR-maintained responding. The primary dependent variable for von Frey testing was withdrawal threshold (in grams). Statistical analysis was accomplished with one- and two-factor Repeated Measures ANOVA as appropriate. A significant one-way or two-way ANOVA was followed by the Duncan post hoc test. Significance was set *a priori* at $p = 0.05$.

RESULTS

Grain vs. Sugar Breakpoints

Figure 1 shows breakpoints (defined as final ratio completed) maintained by grain or sugar pellet reinforcers. Each data point represents mean data across 5 days. The mean final ratio completed for grain-maintained responding was 25.8 and for sugar-maintained responding was 43.8. The mean breakpoint for sugar was significantly greater than the mean breakpoint for grain ($T=3.271$, $df=8$, $p=0.0113$).

Effects of plantar incision on PR responding for grain or sugar

Figure 2 shows the effects of the incisional pain manipulation on operant responding for grain (Left Panel) or sugar (Right Panel) under the PR schedule of reinforcement. Baseline responding was set at 100% and the figure shows %breakpoint for 3hr, 1, 2, and 7 days following incision or anesthesia alone (control). The Left Panel shows that, relative to anesthesia controls, incisional pain produced significant decreases in breakpoints for Post 3hr, and Post-day 1 for rats responding for grain reinforcers (Interaction: $F=4.6$, $df=4$, $p=0.0039$; Condition: $F=4$, $df=4$, $p=0.0084$; incision vs. control: $F=15$, $df=1$, $p=0.0029$). Breakpoints recovered in this group of incision animals by Post-day 7. The Right Panel shows that incisional pain had no effect in rats responding for sugar reinforcers.

Effects of plantar incision on tactile allodynia

Figure 3 shows the effects of the incisional pain manipulation on sensitivity to tactile stimuli using the von Frey test for rats that had a history of responding for grain (Left Panel) or sugar (Right Panel). The Left Panel shows that for rats responding for grain, von Frey thresholds were lower at Post-hr 6, PD1 and PD2, relative to the control anesthesia group (Interaction: $F=3.52$, $df=3$, $p=0.0245$; Time: $F=2.79$, $df=3$, $p=0.0541$; incision vs. control: $F=38.99$, $df=1$, $p=0.0001$). The Right Panel shows that for rats responding for sugar, von Frey thresholds were lower at post-hour 6 relative to the control anesthesia group (Interaction: $F=5.92$, $df=3$, $p=0.0022$; Time: $F=7.65$, $df=3$, $p=0.0004$; incision vs. control: $F=10.98$, $df=1$, $p=0.0062$). There were no differences between incision and anesthesia groups on PD 7.

Effects of diclofenac, on incisional pain-related depression of PR responding and tactile thresholds

Figure 4 shows the effects of the NSAID, diclofenac (5.6 mg/kg, i.p.), on incisional pain-related depression of PR operant responding and tactile thresholds. Figure 4 (Left Panel) shows that diclofenac restored pain-depressed responding at 3 hr post incision for rats responding for grain. Breakpoints for incision + diclofenac were greater than incision alone ($T=4.580$, $df=10$, $p=0.0008$). There were no differences between incision + diclofenac, diclofenac alone, or anesthesia controls. Figure 4 (Right Panel) shows that diclofenac reversed tactile allodynia at 3 hr post incision. Thresholds for incision + diclofenac were greater than incision alone ($T=7.299$, $df=6$, $p=0.0008$). There were no differences between incision + diclofenac, diclofenac alone, or anesthesia controls.

DISCUSSION

The purpose of the present paper was to evaluate the effects of a post-operative pain-like state on progressive ratio (PR) operant responding for food. Given the unique features of PR schedules, this class of operant techniques may provide a way to determine the effects of pain on motivated behavior. The main finding was that an acute incisional pain manipulation decreased progressive ratio-maintained responding and that this pain-related depression of operant behavior varied as a function of reinforcer, and was reversed with a non-steroidal anti-inflammatory drug (NSAID).

The present results are consistent with previous findings that solid and liquid food maintains operant responding in rats over a range of schedules including progressive-ratio schedules, and that sugar has higher reinforcing efficacy than non-sugar reinforcers. For example, Covarrubias and Aparicio [2008] showed that saccharin food pellets maintained higher breakpoints than standard grain pellets in rats, and Sclafani and Ackroff [2003] showed that progressively larger concentrations of sucrose produced progressively higher breakpoints. There is also evidence that sweetened alcohol solutions maintain higher rates of operant behavior than non-sweetened alcohol solutions in rats [Heyman, 1997; Slawecki et al., 1997].

The incision-induced decreases in grain pellet-maintained breakpoints suggest that the incisional pain manipulation can reduce motivation to respond for food. Furthermore, the relationship of the incision effects to a pain-like state is supported by the similar time course of incision effects on grain-maintained responding and tactile sensitivity [Spofford et al., 2009] as well as the reversibility of incision effects by the NSAID analgesic, diclofenac. Together, these results suggest that this incision model of post-surgical pain is sufficient to produce a pain-related decrease in motivation for food-maintained responding in rats. There also exists evidence for other pain models to suppress motivated behavior using standard food-maintained schedules or intra-cranial self-stimulation (ICSS). For example, fixed ratio schedules [Martin et al., 2004, 2005], operant escape [Morgan et al., 2008] and thermal operant techniques [Neubert et al., 2005] have been utilized in innovative ways to quantify preclinical measures of pain and analgesia. Secondly, Negus and colleagues have utilized the ICSS procedure to assess pain-depressed behavior. As an example, this group showed that mu and delta opioids block pain-related depression of ICSS-maintained behavior [Do Carmo et al., 2009; Negus et al., 2012], and that ICSS-maintained responding is mediated, at least in part, by a dopamine mechanism [Leitl et al., 2014; Miller et al., 2015].

The present results also indicate that the magnitude of incision effects on PR responding vary as a function of the reinforcer delivered. The failure of incision to alter sugar pellet-maintained responding could result from at least two features of the sugar pellet reinforcers. First, sugar pellets functioned as higher-efficacy reinforcers than grain pellets in this study, consistent with reports on the relative reinforcing efficacy of sugar [Slawecki et al., 1997; Gosnell et al., 2010; Rasmussen et al., 2010; Sharma et al., 2012], and as a result, the present findings suggest that pain may be more effective at decreasing motivation for weaker, rather than for stronger reinforcers. Second, sugar consumption could produce antinociceptive effects that reduce sensitivity to the noxious stimulus. This possibility is consistent with a

vast literature showing the antinociceptive/analgesic effects of sucrose or glucose in non-human and human subjects [d'Anci et al., 1996; Kanarek et al., 1997; Anseloni et al., 2002; Stevens et al., 2010]. Finally, the shorter duration of tactile allodynia in the sugar pellet group also supports this possibility.

The ability of the NSAID analgesic to restore incisional pain-related depression of PR performance is also consistent with a vast literature showing the analgesic effects of diclofenac and similar NSAIDs against a variety of inflammatory, neuropathic, and post-operative nociceptive states [Spofford et al., 2009; Moller et al., 2012; Leys et al., 2013]. NSAIDs have also been shown to reverse pain-related depression of motivated behavior in the ICSS platform [Leitl et al., 2014] and the conditioned place preference procedure [Rutten et al., 2011]. In the latter example, a range of analgesic drugs, including the NSAID indomethacin, attenuated a carrageenan-induced conditioned place aversion [Cao et al., 2014]. Both CPP and CPA are thought to measure aspects of the affective components of pain-like states in rodents (Johansen and Fields 2004; Xie et al., 2014).

The finding that the acute incision manipulation produced transient but significant decreases in PR performance is consistent with a recent study that utilized a PR schedule to show that CFA and chronic neuropathic pain manipulations decreased breakpoints [Schwartz et al., 2014]. However, the clinical relevance of high-dose CFA and complete nerve-section neuropathic pain procedures is questionable, and thus the construct validity of these chronic pain models is unknown [note: in contrast, face validity and predictive validity of chronic pain models has been reported; see Wang and Wang, 2003; Whiteside et al., 2008]. A more general point is the present finding that the acute pain manipulation produced robust tactile allodynia but only transient decreases in motivated behavior. In other words, motivated behavior appears to be difficult to suppress with conventional and clinically relevant pain stimuli. The reasons for the higher magnitude of behavioral change in the von Frey test of mechanical sensitivity and the weaker magnitude of behavioral change in the PR operant task are unknown, but may be related to differential circuitry recruited by each behavioral assay [Schwartz et al., 2014], the length of exposure to the particular PR schedule [Stafford and Branch, 1998] and/or may be related to operant schedule parameters. For example, PR breakpoints will vary as a function of several motivational variables including food deprivation state, magnitude and type of reinforcer, weight of subject, arithmetic vs. logarithmic PR sequence, and limited hold [Hodos, 1961; Hodos and Kalman, 1963; Baron et al., 1992; Stafford and Branch, 1998; Covarrubias and Aparicio, 2008; Killeen et al., 2009]. A related issue is the recognition that simple reflexive stimulus-response tests like the von Frey test have their own set of limitations [Negus et al., 2006], and the pros and cons of utilizing these stimulus-response measures compared to operant procedures will need to be weighed and may vary according to chronicity of pain state and class of candidate analgesic agent.

Taken together, these findings indicate that incisional pain produces NSAID-reversible decreases in progressive-ratio operant responding, and that the pain-related effects vary as a function of reinforcer type. However, there are at least two limitations to the present findings: (1) progressive ratio operant studies generally require extensive training that can take several weeks, which may explain the relative sparsity of operant procedures in the pain

literature, (2) the pain-related depression of PR-maintained behavior is small and transient (at least in the plantar incision model), suggesting a limited window of utility. Further studies are required to determine the generalizability and utility of PR schedules for baseline assessment of pain and future evaluation of therapeutics.

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Fig. 1. Breakpoints for rats responding for either grain pellets (left bar) or sugar pellets (right bar). ** indicates sugar significantly greater than grain ($p < 0.01$).

Fig. 2.

Effects of plantar incision or anesthesia alone (control) on PR-maintained responding for grain (Left Panel) or sugar (Right Panel). Filled circles represent incision; open circles represent anesthesia alone (controls). *, *** indicates incision decreased relative to anesthesia ($p < 0.05$, 0.001 , respectively).

Fig. 3.

Effects of plantar incision or anesthesia alone (control) on tactile sensitivity as measured with von Frey filaments in rats that respond for grain in operant experiments (Left Panel) or sugar in operant experiments (Right Panel). Filled circles represent incision; open circles represent anesthesia alone (controls). **, *** indicates incision + history of grain reinforcers decreased relative to anesthesia ($p = 0.01, 0.001$, respectively).

Fig. 4.

Effects of the NSAID diclofenac (5.6 mg/kg, i.p.) on plantar incision-depressed breakpoints (Left Panel) and plantar incision-depressed tactile thresholds (Right Panel). X-axes show Condition and Y-axes show % Control Breakpoint (Left) and Tactile Threshold (Right). The first column in each graph represents anesthesia control alone, the second column represents incision alone, the third column represents diclofenac + incision, and the fourth column represents diclofenac alone. **, *** indicates incision + diclofenac increased relative to incision alone ($p < 0.01$, 0.001).