Published in final edited form as:

Pain. 2014 September ; 155(9): 1821–1828. doi:10.1016/j.pain.2014.06.010.

Operant Nociception in Nonhuman Primates

Brian D. Kangas and Jack Bergman

McLean Hospital, Harvard Medical School

Abstract

The effective management of pain is a longstanding public health concern. Morphine-like opioids have long been front-line analgesics, but produce undesirable side effects that can limit their application. Slow progress in the introduction of novel improved medications for pain management over the last 5 decades has prompted a call for innovative translational research, including new preclinical assays. Most current *in vivo* procedures (e.g., tail flick, hot plate, warm water tail withdrawal) assay the effects of nociceptive stimuli on simple spinal reflexes or unconditioned behavioral reactions. However, clinical treatment goals may include the restoration of previous behavioral activities, which can be limited by medication-related side-effects that are not measured in such procedures. The present studies describe an apparatus and procedure to study the disruptive effects of nociceptive stimuli on *voluntary* behavior in nonhuman primates, and the ability of drugs to restore such behavior through their analgesic actions. Squirrel monkeys were trained to pull a cylindrical thermode for access to a highly palatable food. Next, sessions were conducted in which the temperature of the thermode was increased stepwise until responding stopped, permitting the determination of stable nociceptive thresholds. Tests revealed that several opioid analgesics, but not d-amphetamine or ⁹-THC, produced dose-related increases in threshold that were antagonist-sensitive and efficacy-dependent, consistent with their effects using traditional measures of antinociception. Unlike traditional reflex-based measures, however, the results also permitted the concurrent evaluation of response disruption, providing an index with which to characterize the behavioral selectivity of antinociceptive drugs.

Keywords

nociception assay; operant behavior; thermal pull; μ -opioids; opioid efficacy; NOP agonists; squirrel monkey

© 2014 International Association for the Study of Pain. Published by Elsevier B.V. All rights reserved.

Corresponding Author: Brian D. Kangas, Ph.D., McLean Hospital, Harvard Medical School, 115 Mill Street, Belmont, MA 02478, USA, Phone: (617) 855-2148, Fax: (617) 855-2417, bkangas@mclean.harvard.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The authors have no conflicts of interest to report.

INTRODUCTION

The effective management of pain remains an important public health concern. Although morphine-like opioids have long been front-line analgesics for most painful conditions, their clinical utility is restricted by well-recognized liability for side effects, including addiction, respiratory depression, and sedation [3]. Despite the clear need for improved analgesics, progress in the discovery and development of novel candidate medications for pain management over the last 5 decades has been slow. This has provoked well-publicized concern [7; 23], leading to the suggestion that traditional nociception assays might be inadequate for the task of identifying novel candidate medications for pain management and, correspondingly, that new animal models are needed for translational pain research [27; 29; 32; 33; 44].

Currently, analgesiometry in laboratory animals primarily employs thermal, electrical, chemical, and mechanical nociception to assay the anti-nociceptive effects of candidate analgesics [28]. Most commonly used approaches (e.g., tail flick, hot plate, acid-induced writhing, warm water tail withdrawal), use simple spinal reflexes or unconditioned behavioral reactions to nociceptive stimuli. These approaches present both conceptual and experimental limitations. From a conceptual standpoint, simple reflex measures fail to adequately capture any involvement of supraspinal areas of the central nervous system in pain-stimulated responses [5; 8; 31; 44]. Therefore, preclinical animal models of nociception are needed to assay behavioral responses that clearly involve higher-order cortical function. From an experimental standpoint, conventional assays usually rely on a decrease in response (e.g., longer latency to tail flick, decreased writhing, etc). Therefore, it is often difficult to distinguish the role of non specific depression of behavior in antinociception produced by candidate analgesics. For example, morphine has sedative effects over the same range of doses that increase the latency to tail flick, and the interaction of these effects is uncertain.

One way to address the above issues is to establish an index of antinociception that relies on the restoration, rather than suppression, of a response under otherwise nociceptive conditions. Operant-based tasks, unlike assays of reflexive or unconditioned behavioral responses, involve the subject engaging in a volitional response that necessarily involves centrally-mediated processes. Thus, such tasks provide an important alternative approach for the evaluation of candidate analgesics. The utility of an operant-based approach has received some attention [27; 29; 33; 44], few studies have been conducted to examine the effects of anti-nociceptive drugs under operant contingencies. Notable exceptions involve orofacial nociception [2 ;35; 36; 40] and escape or titration methodologies [6; 13; 14; 43; 49].

The present report describes an apparatus and operant procedure to examine both the disruptive effects of nociceptive stimuli on *voluntary* responses in nonhuman primates and behaviorally restorative effects of analgesics. Squirrel monkeys were trained to respond (by pulling down a cylindrical thermode) for a palatable food reinforcer. Next, experiments were conducted in which the temperature of the thermode was increased stepwise until responding stopped. This permitted the determination of nociceptive thresholds, which proved to be highly stable over time and sensitive to varying parameters of the response

requirement. Finally, tests with several types of drugs purported to produce analgesia were conducted to assess their antinociceptive effects under these conditions.

METHOD

Subjects

Four adult male squirrel monkeys (*Saimiri sciureus*) were individually housed in a temperature- and humidity-controlled vivarium with a 12-h light/dark cycle (7am–7pm). Subjects had unlimited access to water in the home cage and were maintained at approximate free-feeding weights by post-session access to a nutritionally balanced diet of high protein banana-flavored biscuits (Purina Monkey Chow, St. Louis, MO). In addition, fresh fruit and environmental enrichment were provided daily. Experimental sessions were conducted 5 days a week (Monday–Friday). The experimental protocol for the present studies was approved by the Institutional Animal Care and Use Committee at McLean Hospital. Subjects were maintained in a vivarium licensed by the U.S. Department of Agriculture and in accordance with the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research [34].

Apparatus

Figure 1 shows a drawing of the operant nociception chamber. A custom-built Plexiglas™ chair measuring 25 cm × 25 cm × 40 cm was housed in a 50 cm × 50 cm × 75 cm sound- and light-attenuating enclosure. A digital video camera was mounted in the inside upper-right corner of the enclosure for real-time session monitoring and an infusion pump (PHM-100-10; Med Associates, St. Albans, VT) was mounted outside the left wall of the enclosure for the delivery of liquid reinforcement. Briefly, each operation of the pump delivered 0.15 ml of 30% sweetened condensed milk (70% water) via Tygon® Microbore tubing (0.40 ID, 0.70 OD; Saint-Gobain Performance Plastics, Paris, France) into an easily-accessible shallow well (2.5 cm diameter) of a custom-designed Plexiglas fluid dispenser (5 cm × 3.5 cm × 1.27 cm) mounted to the inside front wall of the chair. Previous studies in our laboratory have found that a small volume (0.15 ml) of this liquid serves as a powerful reinforcer for squirrel monkeys that is very resistant to satiation even under free-feeding conditions [19]. Three horizontally arrayed white stimulus lights (2.5 cm in diameter) were mounted 50 cm above the enclosure floor, spaced 10 cm apart and centered above the fluid dispenser. A telegraph key was secured to a shelf 15 cm above the stimulus lights, and a custombuilt stainless steel 500w/120v thermode (1.27 cm diameter; 15.24 cm length) with fiberglass leads hung from the telegraph key button via a 5 cm chain. A downward pull of the thermode closed the telegraph key circuit, making an electrical contact that could serve as a response. A temperature sensor (TBC-72.0G, Convectorics, Haverhill, MA) was attached to the upper end of the thermode which also was attached via the fiberglass leads to a 120v, 15amp temperature control unit (Control Console 006-12015, Convectorics, Haverhill, MA). This unit served as a thermostat and controlled the temperature of the thermode with a resolution of ±1°C. All temperature settings and adjustments were made by the experimenter. Other experimental events (i.e., pull detection, operation of stimulus lights, milk delivery) and data collection were controlled by Med Associates (St. Albans, VT) interfacing equipment and operating software.

Procedure

Pull Training—During experimental sessions subjects were seated in the chair. Each subject was trained with response shaping [4], first, to drink from the milk well and, then, to pull the thermode downward to close the telegraph key. Trials began with illumination of the left and right stimulus lights. Thermode pulls with a force of at least 2.78 N closed the telegraph key circuit and were recorded as responses. During initial training, each circuit closure immediately extinguished the left and right stimulus lights and illuminated the center stimulus light for 2 s, delivered 0.15 ml of milk into the well, and was followed by a 10 s intertrial interval (ITI) during which the chamber was darkened. Each training session consisted of 100 trials which were completed in approximately 30 min. After the subject reliably pulled the thermode and closed the telegraph key circuit, the required duration of pull to obtain reinforcement (i.e., duration of continuous telegraph key circuit closure) was increased across successive sessions (0.25, 0.5, 1, 2, and 3 s) until the subject was reliably completing 3 s pulls. Continuous circuit closure during the duration was required and a premature release of the operandum reset that time requirement.

Thermal Threshold Tests—Thermal thresholds were determined during sessions in which subjects were exposed to an ascending sequence of thermode temperatures. Each temperature was evaluated during a 5-trial block, and each trial began with the illumination of left and right stimulus lights (as described above). Completion of the 3 s pull delivered the milk reinforcer, extinguished all stimulus lights within the chamber, and initiated the 10 s ITI. Each 5-trial block was followed by a 2 min blackout period during which all stimulus lights in the chamber were off and the thermode temperature was increased. If 20 s elapsed without a completed response (i.e., limited hold of 20 s), the session was terminated. An ascending sequence of thermal stimulation was used in which the thermode was initially 38°C (approximate body temperature) for the first 5 trials. Following completion of the 5 trials, the thermode temperature increased 2°C during the 2 min blackout and remained at 40°C for the next 5 trials (i.e., 2°C step-size). If all 5 trials were completed, the thermode was again increased by 2°C during the next 2 min blackout period. Blocks of 5 trials at each temperature were used to provide repeated assessment of the subject's performance at that temperature. Thermal thresholds served as the primary dependent measure and were defined as the highest temperature at which the subject completed at least 3 of the 5 trials in a block prior to session termination. An imposed maximum thermode temperature of 60°C was in effect throughout all studies to preclude contact that might produce tissue damage. To insure thermal thresholds were a function of temperature (not number of trials into a session), determinations were periodically conducted from different temperature start points with the proviso that it be at least 2 steps (i.e., 4°C) below the expected thermal threshold and a maximum of 10 step sizes below. Thermal threshold tests under a 3 s pull duration were conducted 5 times in each subject in this manner, i.e., using varying start points.

Following determinations of thermal threshold under a 3 s pull duration, the effects of other pull durations on thermal threshold were assessed parametrically. Subjects were trained to pull the thermode for a variety of durations (0.5, 1, 2, 3, 4, 5, and 6 s). Pull durations <0.5 sec were not studied because 3 of 4 subjects were able to reliably complete 0.5 s responses at the maximum temperature of 60°C. A 6 s pull duration was the longest tested because it

was the longest continuous duration subjects could pull reliably; significant response strain (i.e., increased and variable response latency) was observed in 3 of 4 subjects under a 7 s pull duration requirement. Although all subjects were initially trained and tested under a 3 s pull duration requirement, assessment of other durations were conducted 5 times each in a randomized order across subjects. There were no differential visual stimuli paired with different pull duration requirements; contingent stimulus events (i.e., left and right stimulus lights extinguished, center light illuminated, milk delivered) signaled a completed response under all pull duration requirements.

Drug Effects on Thermal Thresholds—The effects of intramuscular (i.m.) injections of vehicle and a range of doses of several drugs on thermal threshold values were examined next. Drugs included the μ -opioid agonist morphine (0.03–0.32 mg/kg), the mixed-action μ -opioid partial agonist/ δ -opioid antagonist buprenorphine (0.001–0.01 mg/kg), the nociceptin/orphanin FQ peptide (NOP) agonist SCH 221510 (0.003–0.03 mg/kg), the cannabinoid agonist Δ^9 -tetrahydrocannabinol (Δ^9 -THC; 0.03–0.32 mg/kg), and the monoaminergic psychomotor stimulant *d*-amphetamine (0.03–0.32 mg/kg). Drug interaction studies also were conducted to assess antagonism of the antinociceptive effects of morphine and SCH 221510 by pre-session treatment with, respectively, the opioid antagonist naltrexone (0.03–0.32 mg/kg) and the NOP antagonist J-113397 (0.01–0.1 mg/kg). For each drug, conventional cumulative dosing procedures were used to permit the determination of the effects of up to 4 incremental i.m. doses of a drug in single test sessions alone and, for morphine and SCH 221510, after antagonist pretreatment [20; 26; 41]. Briefly, cumulative doses were administered at the beginning of sequential timeout periods that preceded repeated threshold determinations throughout the session. Doses of each drug alone or after pre-session treatment were studied up to those that eliminated responding (see Data Analysis). Each cumulative dose determination was conducted twice with at least 3 intervening days in which thermal control sessions were conducted and baseline values re-established. In addition, during select test sessions, saline vehicle was administered across each of 4 components as described above to verify that the session length and arrangement necessary for cumulative dosing did not produce response fatigue or reinforcer satiation. Finally, the same testing procedures were used to determine cumulative dose response functions for each drug alone under 2 and 5 s pull duration requirements. The sequence of drug experiments varied among subjects.

Drugs— Δ^9 -THC and *d*-amphetamine were provided by the NIDA Drug Supply Program (Rockville, MD); morphine, buprenorphine, and naltrexone were purchased from Sigma Pharmaceuticals (St. Louis, MO); SCH 221510 and J-113397 were purchased from Tocris (Ellisville, MO). Morphine, buprenorphine, *d*-amphetamine, and naltrexone were prepared in saline, whereas Δ^9 -THC, SCH 221510, and J-113397 were initially prepared in a 20:20:60 mixture of 95% ethanol, Emulphor (Alkamuls EL-620; Rhone-Poulenc, Cranbury, NJ), and saline, and further diluted with saline. All drug solutions were refrigerated and protected from light. During test sessions, saline or doses of drug were administered in volumes of 0.3 ml/kg body weight or less by intramuscular (i.m.) injection into calf or thigh muscle.

Data Analysis—Thermal threshold was defined as the highest temperature at which the subject completed at least 3 of the 5 trials, and served as the primary dependent measure of nociception in initial parametric studies of pull duration. Although within-subject threshold values were highly consistent, small differences in threshold values were evident among subjects (see Fig 2). Consequently, data from drug tests were first normalized for individuals by averaging changes from individual subject threshold values across dose determinations, and then presented as group means of change in threshold. A repeated measures one-way analysis of variance (ANOVA) was conducted to evaluate the statistical significance of each drug treatment. When appropriate, ANOVA was followed by a Dunnett's test to evaluate the statistical significance of thermal threshold increases over individual normalized control values. The criterion for significance was set at $p < .05$.

An *in vivo* apparent pK_B analysis of the antagonist effects of naltrexone and J-113397 was conducted for comparison with published values under other assay conditions. The pK_B analysis was conducted using the following equation: $pK_B = -\log [B/(DR - 1)]$, where B is the molar concentration of the antagonist and the dose ratio (DR) is calculated with ED_{50} values (i.e., the dose resulting in 50% of maximum antinociception) of morphine or SCH 221510 alone and after doses of each antagonist pretreatment drug, calculated by interpolation.

RESULTS

Thermal Threshold Tests

A reliable and orderly decrease in thermal threshold was observed as a function of increased pull duration requirements, with a 12°C span in threshold temperature across the full range of pull durations (0.5 to 6 s; Figure 2). Three of four subjects completed 0.5 s pulls at the imposed maximum temperature of 60°C; higher temperatures were not tested to avoid possible tissue damage. An increase in pull duration to 3 s yielded a decrease in average thermal threshold to 51.4°C, whereas the maximal pull requirement, 6 s, resulted in the lowest average thermal threshold of 47.8°C. As noted above, the 6 s duration was the longest tested because three of four subjects were unable to reliably complete continuous 7 s pulls under no-heat conditions, likely due to the relatively large force required (2.78 N) to close the contact of the 10 oz. telegraph key. At least 5 thermal threshold determinations were conducted with each subject, and the reliability of threshold values across repeated determinations was remarkably high for each subject. As indicated by the small variance for mean values in Figures 2 and 3, threshold values under a given pull duration requirement typically differed by no more than one step-size value across individual subjects.

Drug Effects on Thermal Thresholds

Figure 3 presents the effects of saline and dose response functions for each of the 5 agonists. As expected, administration of saline had no effect on thermal thresholds and, importantly, saline injections across 4 components within a test session produced no obvious fatigue or reinforcer satiation, as evident in stable thermal thresholds relative to control. The prototypical μ -opioid agonist morphine produced dose-related and significant increases in thermal threshold values at all three pull durations (2 s: $F=16.89$, $p<.001$; 3 s: $F=16.78$, $p<$

001; 5 s: $F=8.064$, $p<.01$). The lowest dose of morphine was generally without effect, whereas the intermediate doses of 0.1 and 0.32 mg/kg morphine produced comparable and significant increases in threshold values. The highest cumulative dose, 1.0 mg/kg, abolished responding in all subjects and was studied further only in naltrexone-antagonism experiments (see below). The maximally-effective cumulative dose of morphine on thermal threshold, 0.32 mg/kg, also increased mean threshold values by 3°C under a 2 s pull, 4.8°C under a 3 s pull, and 4.3°C under a 5 s pull. Threshold changes in individual subjects were well-represented by group mean data. However, variability in the potency of dose-related effects of morphine under the differing duration response requirements, evident by the largely overlapping error bars in Figure 3a, precluded statistical significance among these differing mean values.

The mixed action μ -opioid partial agonist/ κ -opioid antagonist buprenorphine, like morphine, produced dose-related increases in thermal threshold values (Figure 3b). However, buprenorphine-induced increases in thermal threshold were somewhat lower than those observed for morphine under all response requirements, and only reached statistical significance under the 2 s and 3 s durations (2 s: $F=8.474$, $p<.01$; 3 s: $F=13.36$, $p<.01$; 5 s: $F=3.237$, $p=.08$). The maximally effective cumulative dose of buprenorphine, 0.01 mg/kg increased mean threshold values by 2°C under the 2 s pull condition, 3°C under the 3 s pull condition, and 4°C under the 5 s pull condition. As with morphine, changes in group mean thermal threshold values were representative of changes in individual subjects, and overlapping error bars for mean values among differing response duration requirements indicate no systematic differences in the effects of buprenorphine under these different conditions. Also as observed with morphine, a 0.5 log unit increase in cumulative i.m. dose (to 0.32 mg/kg) produced effects that interfered with performance, eliminating responding in 3 of 4 subjects under all duration response requirements.

The NOP agonist SCH 221510 produced larger dose-related antinociceptive effects than observed with morphine under all duration response requirements tested in the present assay conditions (Figure 3c). Highly significant effects were observed at all three pull durations tested (2 s: $F=17.59$, $p<.001$; 3 s: $F=41.31$, $p<.001$; 5 s: $F=78.11$, $p<.001$). The lowest dose tested, 0.003 mg/kg, was sufficient to produce a significant effect under the 3 s pull duration. The maximally effective cumulative dose of 0.03 mg/kg yielded a 3.6°C increase in mean threshold values under a 2 s pull requirement, a 5.8°C increase under a 3 s pull requirement, and an 8.5°C increase under a 5 s pull requirement. Threshold values obtained following administration of 0.03 mg/kg include maximum possible effects (i.e., completion of trial blocks under the 60°C maximum) in 2 subjects under the 2s pull requirement, and 1 subject under the 3 and 5 s pull requirement. Here again, changes in group mean thermal thresholds were largely representative of the orderly changes observed in individual subjects.

Unlike the opioids morphine and buprenorphine or the NOP agonist SCH 221510, the cannabinoid agonist 9 -THC produced no significant antinociceptive effects in the present studies (2 s: $F=0.2404$, $p=.87$; 3 s: $F=0.5152$, $p=.68$; 5 s: $F=0.9535$, $p=.46$). Small increases in mean thermal threshold values were observed, but only under the 5 s pull requirement (Figure 3d). Moreover, unlike each of the drugs discussed above, mean values represent reliable threshold increases under the 5-sec pull condition in only one of the four subjects

tested with 9 -THC. The psychomotor stimulant *d*-amphetamine produced no evidence of anti-nociception in the present studies (Figure 3e): a wide range of cumulative doses up to those that abolished responding failed to significantly increase thermal threshold in any subject (2 s: $F=0.1579$, $p=.44$; 3 s: $F=1.0$, $p=.99$; 5 s: $F=0.1579$, $p=.92$).

In the final series of experiments, the effects of morphine and SCH 221510 were re-determined in the presence of selected doses of the opioid and NOP receptor-selective antagonists, naltrexone and J-113397, respectively. All tests were conducted under a 3 s response duration requirement. The left panels of Figure 4 show that a range of doses of each antagonist alone did not alter thermal threshold values. However, as indicated in the right panels of Figure 4, the same doses of naltrexone and J-113397 effectively antagonized the effects of, respectively, morphine and SCH 221510 by shifting their dose response functions to the right in a dose-dependent manner. Thus, 0.1 and 0.32 mg/kg of naltrexone produced approximately 7-fold and 20-fold rightward shifts in the morphine dose-response function whereas 0.01 and 0.03 mg/kg of J-113397 produced approximately 6-fold and 30-fold rightward shifts in the SCH 221510 dose-response function. These antagonist effects are reflected in the increased ED_{50} values for each agonist following antagonist treatment (see Table 1). Shifts in group mean dose-response functions were highly representative of the orderly shifts observed in individual subjects. Analyses of the antagonist effects of 0.1 and 0.32 mg/kg naltrexone yielded pK_B values of 7.30 and 7.31, respectively, whereas similar analyses of 0.01 and 0.03 mg/kg J-113397 yielded pK_B values of 8.26 and 8.57, respectively.

DISCUSSION

Over the past several decades, the identification of novel and effective analgesics, especially without marked sedative/stuporific effects, has been a slow and challenging process. In part, this situation simply reflects the paucity of novel types of analgesic compounds that have been identified during this time [7; 23]. As well, however, researchers have identified limitations in *in vivo* assays that may hamper the preclinical evaluation of novel compounds. One limitation that has received considerable attention recently is the inability of current antinociception assays to sufficiently discriminate among candidate analgesics on the basis of unwanted effects that may disrupt ongoing organized behavior [7; 29; 33; 44]. That is, conventional assays [28] are not designed to measure behaviorally-disruptive effects independently of anti-nociception.

With the above consideration in mind, the present apparatus and methods were designed expressly to require a *volitional* operant response under control conditions and, importantly, infer antinociception not by the absence of a response, but rather by its presence. Thus, analogous to the clinical experience of pain that degrades performance, the nociceptive effects of the thermal stimulus was defined as a disruption in ongoing behavior of laboratory subjects. Data from the present studies indicate that this assay yielded highly replicable thermal thresholds in and across individual subjects.

As in numerous studies using traditional analgesiometry, the μ -opioids morphine and buprenorphine produced dose-related antinociceptive effects under the present conditions.

These results indicate that the present assay can be used to evaluate both the disruptive effects of nociceptive thermal stimuli *and* the extent to which a candidate analgesic might restore responding despite nociceptive stimulation. Importantly, the present assay appears to be highly sensitive to both the antinociceptive and suppressant effects of analgesics. For example, the antinociceptive effects of morphine appeared to plateau at the highest doses that could be studied (0.1 and 0.32 mg/kg). The antinociceptive effectiveness of these relatively small doses of morphine under operant contingencies agrees with previous studies with rats and nonhuman primates [35; 43; 49]. Additionally, the antinociceptive effects of morphine and buprenorphine were evident within narrower dose ranges than those reported using conventional assay conditions. For example, 0.32 mg/kg was the largest dose of morphine that provided antinociception in the present study without other effects that precluded successful completion of the required task. This dose of morphine is at least 4 to 10-fold lower than maximally effective doses in squirrel monkeys under the shock-titration assay [1; 14; 38] or stump-tailed macaques under an operant escape procedure [6], and suggests that antinociceptive doses of morphine in other assays also produce behavioral impairment. Overall, the present data reflect a limit in the selective analgesic effects of morphine, due to competing sedative and stuporific effects of morphine that ultimately abolished responding. Following administration of the higher dose of 1.0 mg/kg of morphine, such effects likely abolished responses in all subjects.

Buprenorphine is well-understood to have μ -partial agonist actions, and its antinociceptive effects might be expected to plateau at lower levels than those of the higher efficacy agonist morphine [48]. However, the difference in the elevation of thermal thresholds by buprenorphine and morphine was relatively small under the present assay conditions. Like morphine, the maximally effective dose of buprenorphine under these conditions was at least 30-fold lower than maximally effective doses in squirrel monkeys under the shock-titration assay [13]. The present data suggest that, under operant contingencies requiring restoration of behavioral function as an indication of antinociception, differences between morphine and buprenorphine may be smaller than the full versus partial agonist profiles usually exhibited in preclinical studies. Interestingly, although buprenorphine is considered to have lesser efficacy than morphine, early clinical studies with buprenorphine documented a much longer time course of action but no significant differences in peak analgesia compared to morphine [11; 22]. More recently, these similarities in effectiveness also have been documented in studies of inflammatory pain in human subjects [39].

The NOP receptor (previously called ORL1) has been considered the fourth member of the opioid receptor family, and the development of systemically available NOP agonists (e.g., SCH 221510 [42]) and antagonists (J-113397 [21]) has spurred studies of antinociceptive potential within this drug class. Findings have been mixed to date, with supportive evidence in monkeys using a warm water tail withdrawal assay [25] and failures in rats using the standard tail flick assay [18]. The present findings indicate that under operant conditions, SCH 221510 displayed very effective antinociceptive actions, comparable to, or exceeding those of morphine. For example, the maximally effective dose of SCH 221510 (0.03 mg/kg) produced approximately 2- fold greater antinociception than morphine under the 5 s pull requirement. One explanation for the greater effectiveness of SCH 221510 than

morphine may lie in a greater separation in its potency for producing antinociceptive and response-disruptive effects. Consistent with this idea, Ko and Naughton [24] have demonstrated that intrathecal administration of the endogenous NOP receptor ligand nociceptin/orphanin FQ (N/OFQ), unlike morphine, produced antinociceptive effects in monkeys without evidence of sedation. Alternatively, the greater effectiveness of SCH 221510 than morphine under the present conditions may reflect anxiolytic effects of NOP agonists that have been observed previously in rats [12; 15; 17; 18]. From this perspective, anxiolytic effects of the NOP receptor agonist may be viewed as countering the suppression of organized behavior by the nociceptive stimulus. The possibility of conjoint analgesic/anxiolytic effects of NOP agonists is noteworthy. The prominent role of anxiety in the clinical presentation of pain is well appreciated and researchers have long argued that anxiety and pain may be closely intertwined [10; 46]. The present results with SCH 221510 raise the intriguing possibility that it may be possible to develop novel therapeutics that effectively remediate both aspects of clinical pain.

Pretreatment tests with the selective opioid antagonist naltrexone and the NOP receptor antagonist J-113397 verified that the antinociceptive effects of morphine and SCH 221510 were consequent to, respectively, opioid and NOP receptor-mediated actions. In addition, the pK_B values of naltrexone+morphine and J-113397+SCH 221510 were similar to those obtained in the warm water tail withdrawal assay with nonhuman primates [16; 25], providing additional pharmacological evidence that opioid and NOP receptors, respectively, mediated the antinociceptive effects of these agonists in the present studies.

The effects of the non-opioid drugs *d*-amphetamine and 9 -THC further validate the utility of the present assay procedures. The inability of the psychomotor stimulant *d*-amphetamine to increase thermal threshold was not surprising, as it has no known antinociceptive effects. Nonetheless, the lack of anti-nociceptive effects demonstrates that response rate-increasing effects such as those that are well-documented for *d*-amphetamine [9] are not sufficient to engender increases in thermal threshold.

Although tests with 9 -THC provided some suggestive, albeit non-significant, evidence of antinociception under the present conditions, these effects were evident in only one of four subjects and only when the required response duration was 5 s. These findings are generally consistent with existing literature indicating that 9 -THC is an effective antinociceptive agent under very limited conditions [37]. For example, comparing 9 -THC and heroin in the warm water tail withdrawal assay, Vivian et al. [45] observed that heroin produced a full effect (100%) in rhesus monkeys at both relatively low (50°C) and higher (55°C) water temperatures. In contrast, the maximum antinociceptive effects of 9 -THC were limited under the 50°C test condition (71%) and extremely low (15%) under the 55°C test condition. Collectively, these findings suggest that 9 -THC has minimal antinociceptive effects in nonhuman primates. It is interesting to consider these findings in light of the many reports that 9 -THC produces antinociception in tail flick procedures that are part of the tetrad of assays often used to study cannabinoids in rodents [30]. In those studies, doses of 9 -THC that increase tail flick latency in tetrad studies also markedly decrease locomotor activity and increase catalepsy [47]. Indeed, the magnitude of dose-related 9 -THC effects across

the tetrad are highly correlated, suggesting that response disruption likely plays a key role in ⁹-THC-mediated increases in tail-flick latency.

In summary, results of the present studies are highly consistent with analogous investigations using orofacial pain with rodents [2; 35; 36; 40; 43], illustrating the utility of operant procedures to evaluate nociception and the anti-nociceptive effects of centrally active analgesics. Overall, the antinociceptive effects of drugs in these studies also are in general agreement with previous findings in nonhuman primates [49], although high doses of ⁹-THC previously have been reported to have more consistent antinociceptive effects than observed here [45]. However, fundamental differences between conventional and operant antinociception procedures should be emphasized. In particular, antinociception in the present studies is defined by the restoration of responding that, untreated, would be suppressed by thermal stimulation. The present assay in nonhuman primates uses a positively reinforced response to accomplish this, and, perhaps related to the type of reinforcement, is relatively sensitive to behavioral disruption/suppression produced by analgesics. Such evaluation of the restoration, rather than reduction, of behavior as an antinociceptive endpoint provides insight into the behavioral selectivity of candidate analgesics and, consequently, is an important metric for establishing the range of their clinical, applications. Non-specific behavioral effects attending antinociception can be tolerated under some conditions; however, selective antinociception may be essential to the restoration of function and, under many clinical conditions, is more desirable.

Acknowledgments

We thank Rajeev I. Desai and Carol A. Paronis for comments on a previous version of this manuscript. This research was supported by grants K01-DA035974 (B.D.K.) and R01-DA035857 (J.B.) from the National Institute on Drug Abuse.

REFERENCES

1. Allen RM, Dykstra LA. N-methyl-D-aspartate receptor antagonists potentiate the antinociceptive effects of morphine in squirrel monkeys. *J Pharmacol Exp Ther*. 2001; 298:288–297. [PubMed: 11408554]
2. Anderson EM, Mills R, Nolan TA, Jenkins AC, Mustafa G, Lloyd C, Caudle RM, Neubert JK. Use of the Operant Orofacial Pain Assessment Device (OPAD) to Measure Changes in Nociceptive Behavior. *J Vis Exp*. 2013; 76:e50336. [PubMed: 23792907]
3. Benyamin R, Trescot AM, Datta S, Buenaventura R, Adlaka R, Sehgal N, Glaser SE, Vallejo R. Opioid complications and side effects. *Pain Physician*. 2008; 11:S105–S120. [PubMed: 18443635]
4. Catania, AC. *Learning*. 4th ed. New Jersey: Prentice Hall; 1998.
5. Coghill, R.; Morrow, T. Functional imaging of animal models of pain: high-resolution insights into nociceptive processing. In: Casey, K.; Bushnell, M., editors. *Pain imaging, progress in pain research and management*. Seattle: IASP Press; 2000. p. 211-239.
6. Cooper BY, Vierck CJ Jr. Measurement of pain and morphine hypalgesia in monkeys. *Pain*. 1986; 26:361–392. [PubMed: 3534691]
7. Corbett AD, Henderson G, McKnight AT, Paterson SJ. 75 years of opioid research: the exciting but vain quest for the Holy Grail. *Br J Pharmacol*. 2006; 147:S153–S162. [PubMed: 16402099]
8. Davis, K. Studies of pain using functional magnetic resonance imaging. In: Casey, K.; Bushnell, M., editors. *Pain imaging; progress in pain research and management*. Seattle: IASP Press; 2000. p. 195-210.

9. Dews, PB.; Wenger, GR. Thompson T and Dews PB (Eds) *Adv Behav Pharmacol* (vol 1). New York: New York: Academic Press; 1977. Rate dependency of the behavioral effects of amphetamine; p. 167-227.
10. Dews, PB. What is analgesia?. In: Braude, MC.; Harris, LS.; May, EL.; Smith, JP.; Villarreal, JE., editors. *Narcotic antagonists (advances in biochemical psychopharmacology, vol 8)*. New York: Raven Press; 1974. p. 235-243.
11. Downing JW, Leary WP, White ES. Buprenorphine: a new potent long-acting synthetic analgesic. Comparison with morphine. *Br J Anaesth*. 1977; 49:251–255. [PubMed: 334206]
12. Duzzioni M, Duarte FS, Leme LR, Gavioli EC, De Lima TC. Anxiolytic-like effect of central administration of NOP receptor antagonist UFP-101 in rats submitted to the elevated T-maze. *Behav Brain Res*. 2011; 222:206–211. [PubMed: 21459118]
13. Dykstra LA. Effects of buprenorphine on shock titration in squirrel monkeys. *J Pharmacol Exp Ther*. 1985; 235:20–25. [PubMed: 4045722]
14. Dykstra LA. Effects of morphine, pentazocine, and cyclazocine alone and in combination with naloxone on electric shock titration in the squirrel monkey. *J Pharmacol Exp Ther*. 1979; 211:722–732. [PubMed: 117098]
15. Fernandez F, Misilmeri MA, Felger JC, Devine DP. Nociceptin/orphanin FQ increases anxiety-related behavior and circulating levels of corticosterone during neophobic tests of anxiety. *Neuropsychopharmacology*. 2004; 29:59–71. [PubMed: 14532912]
16. Gerak LR, France CP. Time-dependent decreases in apparent pA₂ values for naltrexone studied in combination with morphine in rhesus monkeys. *Psychopharmacology (Berl)*. 2007; 193:315–321. [PubMed: 17447051]
17. Goeldner C, Spooren W, Wichmann J, Prinssen EP. Further characterization of the prototypical nociceptin/orphanin FQ peptide receptor agonist Ro 64-6198 in rodent models of conflict anxiety and despair. *Psychopharmacology (Berl)*. 2012; 222:203–214. [PubMed: 22249359]
18. Jenck F, Wichmann J, Dautzenberg FM, Moreau JL, Ouagazzal AM, Martin JR, Lundstrom K, Cesura AM, Poli SM, Roevers S, Kolczewski S, Adam G, Kilpatrick G. A synthetic agonist at the orphanin FQ/nociceptin receptor ORL1: Anxiolytic profile in the rat. *Proc Natl Acad Sci USA*. 2000; 97:4938–4943. [PubMed: 10758169]
19. Kangas BD, Bergman J. A novel touch-sensitive apparatus for behavioral studies in unrestrained squirrel monkeys. *J Neurosci Meth*. 2012; 209:331–336.
20. Kangas BD, Delatte MS, Vemuri VK, Thakur GA, Nikas SP, Subramanian KV, Shukla VG, Makriyannis A, Bergman J. Cannabinoid Discrimination and Antagonism by CB1 Neutral and Inverse Agonist Antagonists. *J Pharmacol Exp Ther*. 2013; 344:561–567. [PubMed: 23287700]
21. Kawamoto H, Ozaki S, Itoh Y, Miyaji M, Arai S, Nakashima H, Kato T, Ohta H, Iwasawa Y. Discovery of the first potent and selective small molecule opioid receptor-like (ORL1) antagonist: 1-[(3R,4R)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1, 3-dihydro-2H-benzimidazol-2-one (J-113397). *J Med Chem*. 1999; 42:5061–5063. [PubMed: 10602690]
22. Kay B. A double-blind comparison of morphine and buprenorphine in the prevention of pain after operation. *Br J Anaesth*. 1978; 50:605–609. [PubMed: 352372]
23. Kissin I. The development of new analgesics over the past 50 years: a lack of real breakthrough drugs. *Anesth Analg*. 2010; 110:780–789. [PubMed: 20185657]
24. Ko MC, Naughton NN. Antinociceptive effects of nociceptin/orphanin FQ administered intrathecally in monkeys. *J Pain*. 2009; 10:509–516. [PubMed: 19231294]
25. Ko MC, Woods JH, Fantegrossi WE, Galuska CM, Wichmann J, Prinssen EP. Behavioral effects of a synthetic agonist selective for nociceptin/orphanin FQ peptide receptors in monkeys. *Neuropsychopharmacology*. 2009; 34:2088–2096. [PubMed: 19279568]
26. Lamb RJ, Järbe TUC, Makriyannis A, Lin S, Goutopoulos A. Effects of ⁹-tetrahydrocannabinol, (R)-methanandamide, SR 141716, and d-amphetamine before and during daily ⁹-tetrahydrocannabinol dosing. *Eur J Pharmacol*. 2000; 398:251–258. [PubMed: 10854837]
27. Lascelles BDX, Flecknell PA. Do animal models tell us about human pain? *Pain Clinical Updates*. 2010; 18:1–6.
28. Le Bars D, Gozariu M, Cadden SW. Animal models of nociception. *Pharmacol Rev*. 2001; 53:597–652. [PubMed: 11734620]

29. Mao J. Current challenges in translational pain research. *Trends Pharmacol Sci.* 2012; 33:568–573. [PubMed: 22959652]
30. Martin BR, Compton DR, Thomas BF, Prescott WR, Little PJ, Razdan RK, Johnson MR, Melvin LS, Mechoulam R, Ward SJ. Behavioral, biochemical, and molecular modeling evaluations of cannabinoid analogs. *Pharmacol Biochem Behav.* 1991; 40:471–478. [PubMed: 1666911]
31. Matthies BK, Franklin KB. Formalin pain is expressed in decerebrate rats but not attenuated by morphine. *Pain.* 1992; 5:199–206. [PubMed: 1484716]
32. Mogil JS. Animal models of pain: progress and challenges. *Nat Rev Neurosci.* 2009; 10:283–294. [PubMed: 19259101]
33. Morgan D, Carter CS, DuPree JP, Yezierski RP, Vierck CJ. Evaluation of prescription opioids using operant-based pain measures in rats. *Exp Clin Psychopharmacol.* 2008; 16:367–375. [PubMed: 18837633]
34. National Research Council. Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research. Washington DC: National Academies Press; 2003.
35. Neubert JK, Widmer CG, Malphurs W, Rossi HL, Vierck CJ, Caudle RM. Use of a novel thermal operant behavioral assay for characterization of orofacial pain sensitivity. *Pain.* 2005; 116:386–395. [PubMed: 15982812]
36. Nolan TA, Caudle RM, Neubert JK. Effect of caloric and non-caloric sweet reward solutions on thermal facial operant conditioning. *Behav Brain Res.* 2011; 216:723–725. [PubMed: 20797411]
37. Pertwee RG. Emerging strategies for exploiting cannabinoid receptor agonists as medicines. *Br J Pharmacol.* 2009; 156:397–411. [PubMed: 19226257]
38. Pitts RC, Allen RM, Walker EA, Dykstra LA. Cloccinamox antagonism of the antinociceptive effects of mu opioids in squirrel monkeys. *J Pharmacol Exp Ther.* 1998; 285:1197–1206. [PubMed: 9618423]
39. Ravn P, Secher EL, Skram U, Therkildsen T, Christrup LL, Werner MU. Morphine- and buprenorphine-induced analgesia and antihyperalgesia in a human inflammatory pain model: a double-blind, randomized, placebo-controlled, five-arm crossover study. *J Pain Res.* 2013; 6:23–38. [PubMed: 23359655]
40. Rossi HL, Vierck CJ Jr, Caudle RM, Neubert JK. Characterization of cold sensitivity and thermal preference using an operant orofacial assay. *Mol Pain.* 2006; 2:37. [PubMed: 17166284]
41. Spealman RD. Discriminative-stimulus effects of midazolam in squirrel monkeys: Comparison with other drugs and antagonism by Ro 15-1788. *J Pharmacol Exp Ther.* 1985; 235:456–462. [PubMed: 2932547]
42. Varty GB, Lu SX, Morgan CA, Cohen-Williams ME, Hodgson RA, Smith-Torhan A, Zhang H, Fawzi AB, Graziano MP, Ho GD, Matasi J, Tulshian D, Coffin VL, Carey GJ. The anxiolytic-like effects of the novel, orally active nociceptin opioid receptor agonist 8-[bis(2-methylphenyl)methyl]-3-phenyl-8-zabicyclo[3.2.1]octan-3-ol (SCH 221510). *J Pharmacol Exp Ther.* 2008; 326:672–682. [PubMed: 18492950]
43. Vierck CJ, Acosta-Rua A, Nelligan R, Tester N, Mauderli A. Low dose systemic morphine attenuates operant escape but facilitates innate reflex responses to thermal stimulation. *J Pain.* 2002; 3:309–319. [PubMed: 14622755]
44. Vierck CJ, Hansson PT, Yezierski RP. Clinical and pre-clinical pain assessment: are we measuring the same thing? *Pain.* 2008; 135:7–10. [PubMed: 18215466]
45. Vivian JA, Kishioka S, Butelman ER, Broadbear J, Lee KO, Woods JH. Analgesic, respiratory and heart rate effects of cannabinoid and opioid agonists in rhesus monkeys: antagonist effects of SR 141716A. *J Pharmacol Exp Ther.* 1998; 286:697–703. [PubMed: 9694923]
46. Von Korff M, Simon G. The relationship between pain and depression. *Br J Psychiatry.* 1996; 168:101–108.
47. Wiley JL, Smith FL, Razdan RK, Dewey WL. Task specificity of cross-tolerance between Delta9-tetrahydrocannabinol and anandamide analogs in mice. *Eur J Pharmacol.* 2005; 510:59–68. [PubMed: 15740725]
48. Woods, JH.; France, CP.; Winger, GD. Behavioral pharmacology of buprenorphine: Issues relevant to its potential in treating drug abuse. In: Blain, JD., editor. *Buprenorphine: An Alternative for Opiate Dependence*. National Institute on Drug Abuse Research Monograph 121.

DHHS Pub. No. (ADM) 92-1912. Washington, DC: Supt. of Docs., U.S. Govt. Print. Off; 1992. p. 12-27.

49. Yeomans DC, Cooper BY, Vierck CJ Jr. Effects of systemic morphine on responses of primates to first or second pain sensations. *Pain*. 1996; 66:253–263. [PubMed: 8880848]

Summary

We discuss validation of an operant model of nociception with nonhuman primates that effectively characterized the behavioral selectivity of analgesic drugs.

Figure 1.
Schematic of the operant nociception chamber. See apparatus section for additional details.

Figure 2.
Group mean ($\pm$ SEM) thermal thresholds ($^{\circ}$ C) under various pull duration requirements.

Figure 3.

Dose-response functions of group mean (±SEM) changes from baseline thermal thresholds of cumulative doses of morphine (Panel a), buprenorphine (Panel b), SCH 221510 (Panel c), ⁹-THC (Panel d), and *d*-amphetamine (Panel e) under 2, 3, and 5 s pull duration requirements.

Figure 4.

4 Dose-response functions for (a) naltrexone, (b) cumulative doses of morphine alone and following pretreatment with naltrexone, (c) J-113397, and (d) cumulative doses of SCH 221510 alone and following pretreatment with J-113397. All tests were conducted under a 3-second pull duration requirement.

Table 1

ED₅₀ values for morphine and SCH 221510 administered alone (baseline) or after pretreatment with various doses of naltrexone and J-113397, respectively. Values are given in milligrams per kilogram

Naltrexone	ED ₅₀	J-113397	ED ₅₀
Morphine Alone	0.04	SCH 221510 Alone	0.004
0.1	0.30	0.01	0.024
0.32	0.88	0.03	0.121