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Intranasal Ketamine for Acute Pain: Behavioral and Neurophysiological Safety Analysis in Mice

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ABSTRACT

Background: Subanesthetic ketamine has been used for treatment-resistant depression and is popular as an opioid-sparing agent.
Objective: The present study aimed to investigate the dose-dependent antinociceptive effect of intranasal ketamine (INK) along with behavioral and neurophysiological safety in mice.
Methods: Antinociceptive efficacy was evaluated in the terms of thermal nociceptive response and formalin test. The safety studies were carried out separately in healthy mice using telemetry-based cortical electroencephalography, hemodynamic changes, and spontaneous behavioral functions, including anxiety, stereotypic movement, and locomotor functions.
Results: INK administration significantly augmented the thermal nociceptive threshold and alleviated the pain response in the tonic phase of the formalin test. The results showed the dose-independent effectiveness of ketamine for thermal nociceptive responses because there were no significant differences among different INK dose groups. Behavioral safety analysis using the open field exploratory test revealed no significant effect of INK on anxiety-like functions in healthy mice. However, INK mice showed significantly more stereotypic movement but slower locomotor activities. The electroencephalography signal power spectrum density analysis revealed no significant changes by INK administration except a lower value in the α range. No significant changes were reported in heart rate, diastolic blood pressure, or systolic blood pressure at the higher dose equivalent used in the pain model.
Conclusions: The study demonstrated the behavioral and neurophysiological safety of INK, although it had a mild sedative effect. Therefore, INK is suggested as a potentially safe candidate for the management of acute pain. (Curr Ther Res Clin Exp. 2021; 82:XXX–XXX)

Introduction

Acute pain management is very challenging in the austere prehospital environment, wilderness, and battlefield. In resources-deprived areas, it is difficult to adhere to the World Health Organization analgesic ladder. Due to the serious cardiorespiratory complications of opioids, nonsteroidal anti-inflammatory drugs (NSAIDs) are the primary choice for mild-to-moderate pain in surgical settings. However, NSAID poisoning of platelet function may worsen hemostasis in cases of excessive bleeding.[1] Although selective cyclooxygenase-2 inhibitors could be a suitable alternate, both the NSAID and cyclooxygenase-2 inhibitors may cause renal complications in dehydrated patients experiencing austere environments.[2,3]

Ketamine is widely used as an anesthetic agent in both experimental animal studies and clinically. Ketamine represents a unique property to induce electrophysiological dissociation between the thalamocortical and limbic systems.[4] It is a noncompetitive antagonist to the phencyclidine site of the N-methyl-D-aspartate (NMDA) receptor for the excitatory neurotransmitter glutamate. Besides an anesthetic, ketamine is being used for several clinical conditions like chronic obstructive airway disease, opioid-induced hyperalgesia, status epilepticus, and depression.[5,6] The complex mechanism of action and diverse function of ketamine is attributed to its interaction with numerous receptors and subreceptor systems, including nicotinic, muscarinic, cholinergic, monoaminergic, and opioid receptors.[7] The subanesthetic doses of ketamine alone

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or in combination with other agents like midazolam has been used in relieving pain associated with burn dressing, procedure-related pain in pediatric oncology patients, and postoperative pain.[8]

Therefore, the ketamine in a subanesthetic dose may be a suitable option for acute pain management in surgery settings. However, pain management in an austere setting carries several unique challenges like the overdose safety, size of the kit, and self-usability of analgesics. Therefore, the intranasal route can be a convenient and effective route for the self-administration of drugs. Besides being simple and noninvasive, the intranasal route is very suitable for patients with nausea or vomiting. The nasal cavity is a unique portal for drug delivery to the brain through the trigeminal and olfactory pathways. The nasal route is easy and simple for self-administration of drugs to reach the brain parenchyma by bypassing the major physiological barriers and hepatic/gastrointestinal first pass. Therefore, in light of the major challenges presented by austere settings in acute pain management, the present study aimed to investigate the dose-dependent antinociceptive efficacy of intranasal ketamine in a rodent model of acute pain. Ketamine was further evaluated for behavioral and neurophysiological safety.

Materials and Methods

Animals

C57BL/6J female mice weighing ~22 g and aged 2 months were issued from an in-house inbred colony. Mice were acclimatized for 1 week to a behavioral lab environment under a 12/12 light-dark cycle, temperature of 22°C (±1°C), and free access to food and water. Behavioral lab conditions and acclimatization schedule were based on our earlier study.[9] All experiments were performed with the approval of the institute's animal ethics committee (8/GO/RBi/S/99/CPCSEA).

Experimental design

Animals were divided into 4 groups, n = 7 each. The sample size was based on earlier recommendations and suggestions.[10] Group I: control, group II through group IV: 20 mg/kg, 5 mg/kg, and 1 mg/kg ketamine, respectively. Ketamine was administered intranasally (INK) using a micropipette. Ketamine prescription for animal research purposes was obtained from a government-authorized registered veterinarian. The clinically used ketamine formulation ketamine hydrochloride (Indian Pharmacopeia) 50 mg/mL was procured from a reputable drug store and verified through batch number verification. The desired concentration was obtained using phosphate-buffered saline. The final volume for INK was 10 µL. Control mice were administered an equal volume of intranasal saline. Mice cages were randomly assigned coded labels and the experimenter was blind to treatment groups. Mice were then tested for spontaneous behavioral functions. Similar separate groups of mice were used to assess the dose-dependent efficacy of ketamine against the thermal nociceptive threshold. In view of the 3R (Replacement, Reduction and Refinement) principles of judicious animal use, the formalin test was carried out at only the lower dose level (1 mg/kg) and compared with the saline administered mice. Similarly, hemodynamic changes and electroencephalography analysis were carried out at the higher dose level following 30 minutes of INK administration. The lowest dose selected in the present study was based on the earlier finding.[11]

Behavioral assays

Thermal nociception

For the quantification of the central analgesic effect of ketamine, the thermal nociceptive threshold of mice was measured using a hot-plate test. After 30 minutes of drug administration, mice were placed individually on a hot plate maintained at 52°C (±1°C), and reaction time was recorded. Reaction time was considered as latency to flick or lick the paw. Mice were not tested beyond cutoff time (20 second).

Formalin test

A formalin test was performed as described earlier[12] with minor modifications. Precisely 20 μL 2% formalin solution was injected into the dorsal surface of the left hind paw of each mouse (after 15 minutes of ketamine administration), using a 26-gauge needle. Immediately thereafter, the mouse was kept in a transparent cage and the video was recorded. The time spent licking or biting the affected paw during the first phase (0–5 minutes) and a second phase (15–20 minutes) was measured as an indicator of pain.

Spontaneous behavioral functions

The spontaneous behavioral functions of mice were recorded using an automated behavioral monitoring system Opto-Varimex version 4.93 (Columbus Instruments, Columbus, Ohio) as described previously with minor modifications.[13] Mice were placed for 5 minutes in test arena units ($35 \times 29 \times 10$ cm) equipped with infrared sensors and the analysis was carried out for various parameters: total distance (in centimeters), stereotypic movements (in seconds), distance traveled in the center (in centimeters), and resting time in the XY plane. The behavioral observation was carried out 30 minutes following drug administration.

Hemodynamic measurements

INK-induced changes in hemodynamic parameters were recorded using CODA NIBP System (Kent Scientific, Torrington, Connecticut) according to technical instruction provided. For the initial 3 days, mice were acclimatized to the apparatus to eliminate possible restraining stress-induced changes in hemodynamic parameters. Following the acclimatization, there was a substantial decrease in standard deviation, and values of different parameters attained steady-state (see the **Supplemental Figure** in the online version at doi:10.1016/j.curtheres.2021.100627). On testing day, mice were kept in a restraining tube on a pre-heated warming platform maintained at 37°C after 30 minutes of ketamine (20 mg/kg) administration. Occlusion cuff and volume pressure recording cuffs were placed on the tail of mice and measurements were taken. Finally, the values considered true by CODA NIBP software were accepted and averaged for each animal. Data were analyzed for the quantification of systolic blood pressure, diastolic blood pressure, and mean blood pressure along with heart rate.

Electroencephalography

The ketamine-induced changes in the cortical biopotentials were recorded using a wireless electroencephalography (EEG) system. For EEG transmitter implantation, mice were deeply anesthetized using the cocktail of ketamine (90 mg/kg) and xylazine (10 mg/kg) at a final volume of 0.1 mL/25 g (intraperitoneal injection). Mice were kept on a heating pad (37°C) during the whole microsurgical procedure. Briefly, a midline scalp incision was made from the eyes to the neck to expose the skull. Two tiny burr holes, 1 at each side, were trephined manually on the skull over the parietal lobe of both the hemisphere about 1.5 mm posterior to bregma and 2.0 mm lateral to the sagittal suture. Two screws were placed in the holes while touching the surface of the dura and connected to the transmitter electrodes. Finally, the assembly was secured to the parietal bone using dental acrylic cement and topical tissue adhesive (n-butyl-2-cyanoacrylate). The transmitter was placed subcutaneously at the back of the mice. After the surgery, mice were allowed to recover for 7 days before the acquisition of

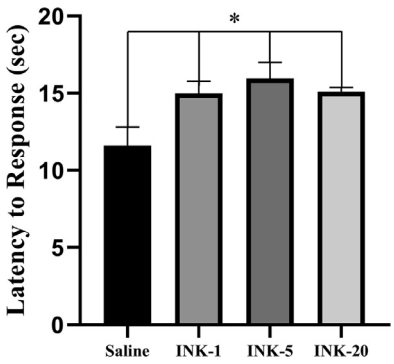

Figure 1. Thermal nociceptive response of mice as the withdrawal reflex was quantified in the hot-plate test. Data were analyzed using a 1-way ANOVA followed by Dunnett's multiple comparisons test and presented as mean and standard error of the mean (indicated by vertical bars) (n = 7). *Significant difference at $P < 0.05$.

EEG data. EEG data acquisition was made through a wireless stellar telemetry system (TSE Systems GmbH, Bad Homburg, Germany). EEG data were recorded continuously in packets of 10 seconds at the time interval of 5 minutes for 30 minutes at the rate of 200 samples per second before (baseline) and after INK administration. Data were analyzed using the Acqknowledge version 5.0 (Biopac Systems Inc, Goleta, California) software provided with the stellar telemetry system.

Statistical analysis

Data were analyzed using GraphPad Prism 8 Software (GraphPad Software Inc, La Jolla, California) and presented as mean and standard error of the mean. Statistical significance was computed using a 1-way ANOVA followed by Dunnett's multiple comparisons test (wherever required). For analyzing data of CODA NIBP and formalin test, Student t test was applied. Statistical significance was taken at 95% CIs ($P \leq 0.05$).

Results

Pain and nociceptive threshold

The nociceptive threshold of mice to the thermal stimuli was measured at different doses of INK (20 mg/kg, 5 mg/kg, and 1 mg/kg) using a hot-plate test (Figure 1). The 1-way ANOVA showed a significant main effect of groups on latency to response ($F_{3,24} = 4.327$; $P = 0.0183$). Post hoc analysis revealed a dose-independent increase in latency to reaction time in INK-treated mice compared with the control ($P < 0.05$). Although INK was effective at all the doses tested, no significant difference in the nociceptive threshold was observed among different doses of ketamine.

The analgesic effect of INK was further evaluated at the lowermost dose (1 mg/kg) using the formalin test. The injection of 2% formalin on the dorsal paw surface produced a biphasic behavioral change in the term of licking/biting of the affected paw both in control and ketamine-treated mice (Figure 2). Data were analyzed for both the acute (0–5 minutes) and tonic phase (15–20 minutes) of nociceptive response following the formalin injection. INK (1 mg/kg) markedly inhibited the formalin-induced pain in the tonic phase as indicated by a significant decrease ($P < 0.05$) in the time spent licking/biting. Results showed that the formalin-induced nociceptive response was nearly 4 times lesser in ketamine-treated mice.

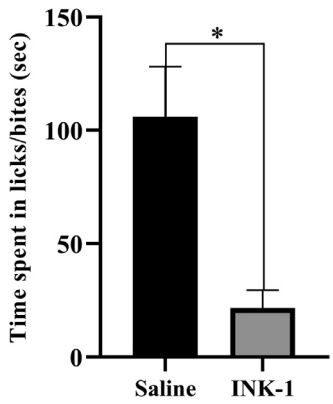

Figure 2. Antinociceptive effects of intranasal ketamine (INK) in the tonic phase of the formalin test. Results were analyzed using the Student t test. Data are represented as mean and standard error of the mean (n = 7). *Statistical significant comparison at $P < 0.05$.

Spontaneous behavioral functions

INK-induced changes in the spontaneous behavioral functions were evaluated in an open field exploratory test arena using infrared sensors. The values were quantified in the terms of total locomotor activities, stereotypic movement, and anxiety-like behavior. INK administration at different doses resulted in significant changes in various spontaneous behavioral functions. One-way ANOVA showed a significant main effect of factor drug doses ($F_{3,24} = 6.582$; $P = 0.0031$) on locomotor activity (Figure 3A). Post hoc analysis showed a significant decrease in locomotor activity of treated groups compared with the control group ($P < 0.001$; 5 mg/kg and 1 mg/kg and $P < 0.05$; 20 mg/kg). The activity data showed a significant main effect of treatment ($F_{3,24} = 4.163$; $P = 0.02$) on resting time (Figure 3B). Post hoc analysis showed a significant ($P < 0.05$) increase in the resting time at a lower dose (1 mg/kg) but not at other higher doses.

Anxiety-like behavior was accessed in the term of center exploratory activity in the open field test arena. One-way ANOVA showed no significant main effect of treatment ($F_{3,24} = 1.261$; $P = 0.3316$) on distance traveled in the center (Figure 4A). Further, stereotypic movements are considered as motor responses without a specific purpose or goal. One-way ANOVA showed a significant main effect of treatment ($F_{3,24} = 4.211$; $P = 0.0164$) on time spent in stereotypic movements (Figure 4B). Post hoc multiple comparisons revealed a significant increase in stereotypic movements in treated groups at every dose in comparison to the control group ($P < 0.05$). No significant dose-dependent effect of INK was observed on the stereotypic movements.

Hemodynamic parameter changes

INK-induced hemodynamic changes were evaluated in the terms of systolic pressure, diastolic pressure, mean blood pressure, and heart rate in the restraint mice. Because restraint stress itself causes tremendous changes in the hemodynamic parameters, the mice were acclimatized to the condition for 3 days before the test session. Following the acclimatization, when mice attained the steady-state in their respective hemodynamic values (see the **Supplemental Figure** in the online version at doi:10.1016/j.curtheres.2021.100627), INK test was carried out on the fourth day. The effect of INK on hemodynamic parameters was evaluated at the highest dose. The effect of INK on the systolic pressure, diastolic pressure, mean blood pressure, and heart rate was significantly not different from the control mice (Figure 5A–5D).

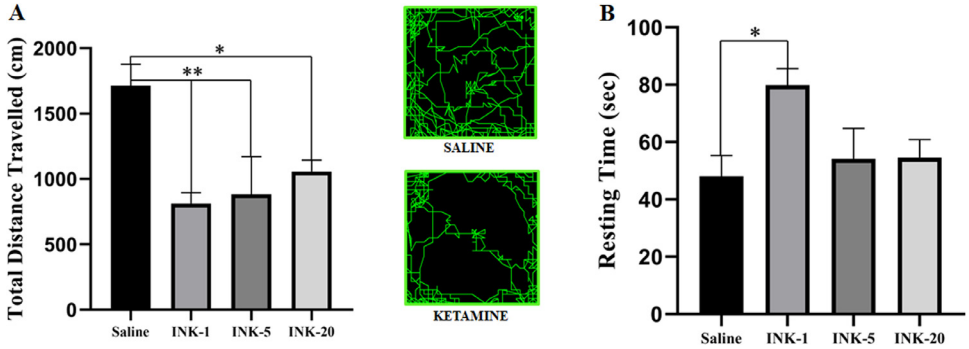

Figure 3. The total locomotor activity was expressed as (A) distance traveled in the open field exploratory test arena and along with track plots generated through IR beam crossing over (representative images) and (B) resting time. Data were analyzed using 1-way ANOVA followed by Dunnett's multiple comparisons test and presented as mean and standard error of the mean (n = 7). *Significant difference at $P < 0.05$. **Significant difference at $P < 0.001$.

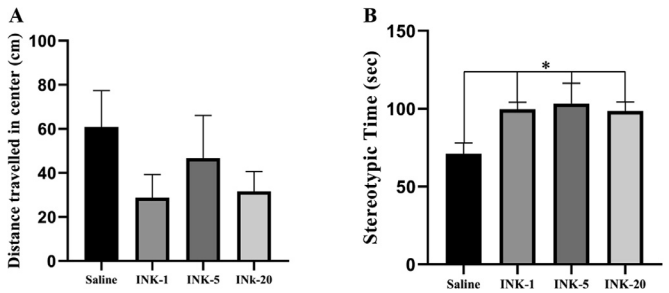

Figure 4. Intranasal ketamine (INK)-induced changes in anxiety-like behavior and stereotypic movements. (A) Anxiety-like behavior depicted as total distance traveled in the center of open field. (B) Time spent in stereotypic movements. Results were analyzed using 1-way ANOVA followed by Dunnett's multiple comparisons test. Data are presented as mean and standard error of the mean (n = 7). *Significant comparison at $P < 0.05$.

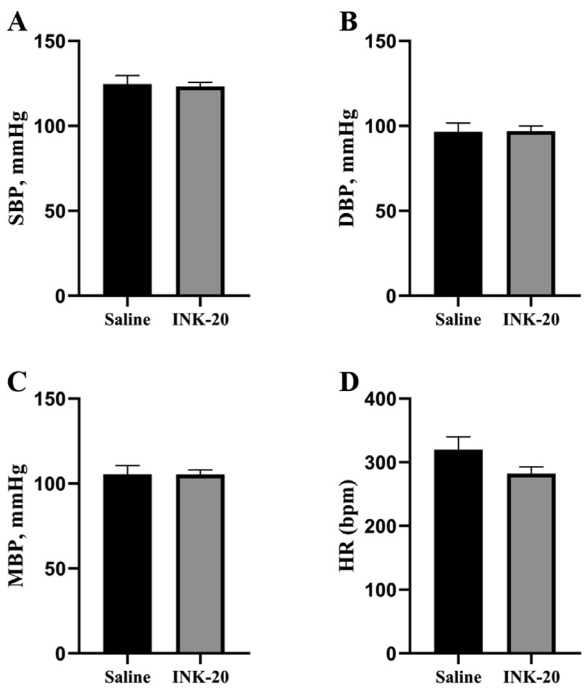

Figure 5. Intranasal ketamine (INK)-induced changes in (A) systolic blood pressure (SBP), (B) diastolic blood pressure (DBP), (C) mean blood pressure (BP), and (D) and heart rate (HR) following 30 minutes of drug administration. Results were analyzed using the Student *t* test. Data are presented as mean and standard error of the mean (n = 7).

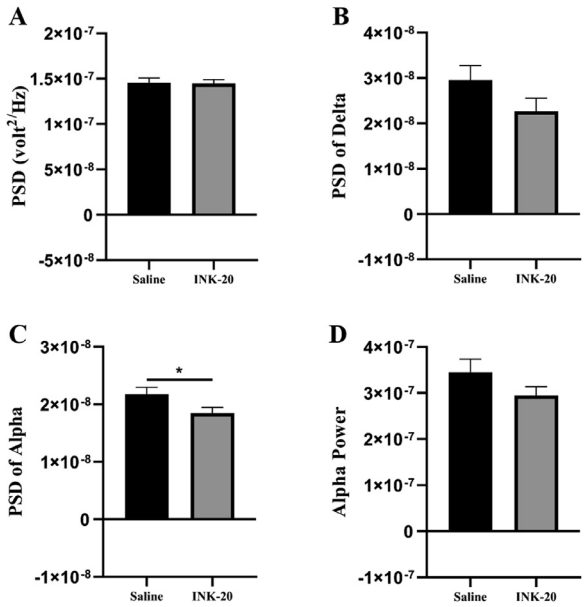

Figure 6. The cortical electroencephalogram (EEG) signals were recorded in mice using the radiotelemetry system in the freely moving mice. (A) Gross power spectral density (PSD) incomplete frequency band (1–100 Hz). (B) PSD values in the δ band. (C) PSD values in the α band and D. Values of α power. Data are presented as mean and standard error of the mean. *$P < 0.05$.

Cortical electroencephalogram

The cortical EEG signals were recorded in mice using the radiotelemetry system in the freely moving mice (Figure 6). The power spectral density (PSD) analysis revealed no significant effect of ketamine on the intensity in the full frequency band as well as the δ band. The PSD value in the α band was significantly reduced following INK administration (20 mg/kg) compared with the control (baseline) (Figure 6C). However, no significant changes in α power were reported following the INK administration.

Discussion

The results showed the dose-independent effectiveness of INK on the nociceptive threshold for thermal stimulus. The lack of increasing analgesic effect at the higher ketamine dose was also reported in an earlier clinical study using subdissociative dose of INK for moderate-to-severe pain in adult emergency department patients.[14] Although it is beneficial in avoiding the overdose side

effect, the possible reason might be an active mucociliary clearance and short retention time. Moreover, dose-independent effectiveness might be also due to complete receptor saturation at a lower dose. The recommended intranasal drug volume for mouse subjects is only 10 µL for a total nasal volume of 0.03 mL and a mean nasal epithelial surface of 2.8 cm^2.[15,16] Despite a smaller surface area, INK was very effective even at the lower dose. The low-dose effectiveness of INK is attributed also to a proportionately bigger olfactory region in mice, which is 50% of the nasal cavity in comparison to 10% in humans.[17] The effective analgesic dose of INK in previous clinical studies represents a very wide therapeutic window ranging from 0.2 to 50 mg/kg for different pain states.[18–21] Extrapolation of the human equivalent dose of INK for mice falls in a rather broader range of 2.46 to 612 mg/kg. In the current study, the antinociceptive effect of ketamine was further assessed by the formalin test. INK administration significantly alleviated the pain-response in the tonic phase of the formalin test even at a 1 mg/kg dose. An earlier study also reported a similar trend where INK was not effective in the first phase of the orofacial formalin test.[8] We reported that a ketamine dose as high as 20 mg/kg was physiologically safe in terms of heart rate, diastolic blood pressure, and systolic blood pressure. Although ketamine is extensively used in combination with xylazine for preclinical surgical processes, no sufficient data are available for hemodynamic changes due to ketamine alone, especially for intranasal dose.[22] Earlier clinical findings suggest no significant effect of an intravenous dose of ketamine (3 mg/kg) on blood pressure, heart rate, and respiratory rate.[23]

We have also reported spontaneous behavioral functions, including anxiety, stereotypic movement, and total locomotor functions. The results showed a significant decrease in locomotor activities at an early phase (5 minutes) of open field exploration. However, total locomotor activities for a 10-minute test paradigm were not significantly influenced by INK. No significant effect of INK was reported on anxiety-like functions as evaluated by center exploratory tendencies in open field tests. The ketamine-administered mice showed a significantly more stereotypic movement but higher resting time in the low-dose group. Therefore, the doses of INK administered proved to be behaviorally safe and in the range of subdissociative dose. Being a noncompetitive NMDA receptor antagonists, the reported effect of ketamine in healthy human subjects resemble both positive and negative symptoms of schizophrenia.[24,25] However, ketamine in subdissociative doses is suggested to be behaviorally safe.[26] Moreover, ketamine has proven effective in treating affective dysfunctions like depression.[27,28] Nevertheless, comprehensive preclinical behavioral analysis following low-dose INK administration is not available.

Furthermore, we recorded the cortical EEG using the radiotelemetry system in freely moving mice. There was a significant decrease in the EEG signal PSD value in the α band but not in other frequency bands. The decreased α PSD value corroborates with a deceased locomotor function in the early phase of open field exploration. In an earlier study, electrophysiological recordings in mice with ketamine and other NMDA receptor antagonists resembled the EEG features of schizophrenia.[29] Clinical findings suggest dose-dependent increases in δ PSD. Ketamine dose ranging from 0.2 to 0.5 mg/kg is reported to increase the θ and decrease α in comparison to baseline values.[30] Although contrary to our finding, the previous studies are not an exact match due to the difference in method, route of administration, and the dose of ketamine. Because we employed a wireless radiotelemetry system in freely moving mice, it offers an advantage to attain precision in contrast to classical procedures such as tethered systems or jacket systems containing recorders. Therefore, the present study represents a unique finding of cortical biopotential changes in freely moving mice following INK administration.

Conclusions

Our results conclusively demonstrate the behavioral and neurophysiological safety of INK at an effective dose for acute pain management. It is suggestive of a potential candidate for acute pain management, especially in an austere setting like wilderness or surgery environments. Nevertheless, further study is warranted, especially in relation to the development of suitable intranasal formulation and device for self-administration in austere settings.

Conflicts of Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

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K. Manda conceived, designed, analyzed the data, and wrote the manuscript. N. Goswami designed and carried out experiments, analysis, and contributed to the manuscript. M. Aleem contributed to conduct of the experiments and analyzed the data.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.curtheres.2021.100627.

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