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Research paper

Establishing an animal model for National Acupuncture Detoxification Association (NADA) auricular acupuncture protocol

Vasanth Kattalai Kailasam[a,b], Preeti Anand[a], Zara Melyan[a,*,1]

[a] Department of Anesthesiology, Columbia University Medical Center, New York, NY, USA
[b] Department of Psychiatry, Harlem Hospital Center, Columbia University Medical Center, New York, NY, USA

HIGHLIGHTS

- NADA acupuncture reduces morphine-induced locomotor sensitization.
- NADA acupuncture prevents development of tolerance following chronic morphine treatment.
- NADA auricular acupuncture can improve adverse effect profile of morphine in a rat model.

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ABSTRACT

The use of opioids in the treatment of chronic pain has increased dramatically in the past few decades making them one of the most commonly prescribed medications in the US. However, long-term use of opioids is limited by development of tolerance (decreased antinociceptive efficacy) and opioid-induced hyperalgesia – paradoxical sensitization to noxious (hyperalgesia) and non-noxious (allodynia) stimuli. Novel adjunctive therapies are needed to increase the efficacy and prolong the duration of action of opioids in chronic pain treatment.

Acupuncture is often used as an adjunct therapy for the treatment of symptoms induced by non-clinical use of opioids. The National Acupuncture Detoxification Association (NADA) auricular acupuncture protocol is the most common form of acupuncture treatment for substance abuse. The standardized, easy to use and virtually painless procedure make it an attractive complementary treatment option for patients suffering from opioid-induced adverse effects. Clinical trials designed to test the efficacy of the NADA protocol yielded contradictory results. The mechanism by which NADA acupuncture could serve as a successful treatment remains unknown. Therefore, establishing an animal model of NADA acupuncture can provide a tool for investigating the efficacy and cellular mechanisms of NADA treatment.

Previous studies have shown that repeated morphine administration in rodents can produce locomotor sensitization and reduce analgesic potency of a challenge dose of morphine, indicating development of morphine tolerance. Here we show that NADA acupuncture treatment can both reduce morphine-induced locomotor sensitization and prevent the development of morphine tolerance in rats, thus validating a new model for NADA acupuncture studies. Our data provides support for evidence-based use of NADA acupuncture as a new adjunctive approach that can potentially improve the side-effect profile of morphine and other prescription opioids.

1. Introduction

The National Acupuncture Detoxification Association (NADA) auricular acupuncture protocol is the most common form of acupuncture treatment for substance abuse in the US and Europe [1,2]. This protocol includes five ear acupuncture points and is used to relieve opioid withdrawal symptoms, prevent cravings, and increase patient participation rates in long-term treatment programs [3]. According to the Substance Abuse and Mental Health

* Corresponding author at: Department of Anesthesiology, Columbia University Medical Center, 630 West 168th Street, P.H. 5-505, New York, NY 10032, USA.
E-mail address: zara.melyan@yahoo.co.uk (Z. Melyan).
[1] Current affiliation: The Lang Research Center, New York Presbyterian Queens, Flushing, NY, USA.

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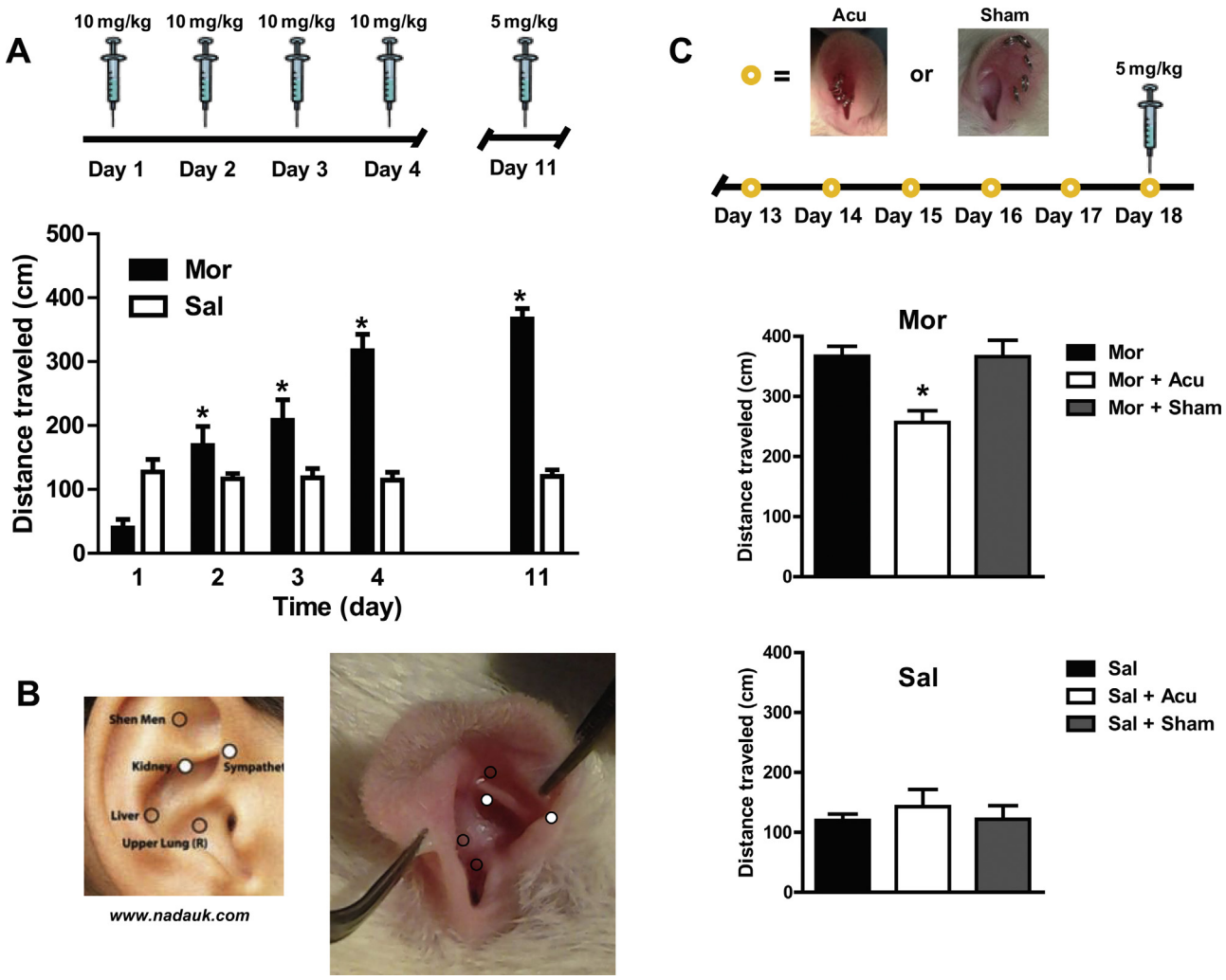

Fig. 1. NADA acupuncture reduces morphine-induced locomotor sensitization in rats. (A) Locomotor activity measurements in morphine (Mor) and saline (Sal) groups showing that repeated doses of morphine produce locomotor sensitization (an increase in distance travelled) * $p < 0.05$, vs baseline (day 1), paired *t*-test, n = 12. (B) NADA acupuncture point localization. (C) Summary histogram showing locomotor activity measurements before (day 11) and after 6 NADA or sham acupuncture sessions (day 18). NADA acupuncture alleviated sensitization produced by morphine (n = 6, p = 0.0022, F = 6.280, one-way ANOVA). No significant change was observed in saline-treated rats.

Services Administration report of 2000 from the US government [4], more than 700 publicly licensed addiction treatment programs include acupuncture as a therapeutic tool. A more recent estimate determined that at least 1500 addiction programs worldwide use acupuncture [5]. The actual number of health providers using NADA acupuncture treatment is likely much higher since these estimates do not include private acupuncture clinics. In fact, NADA estimates that there are more than 2000 clinics worldwide. Globally more than 25,000 health workers have completed NADA acupuncture training [6].

Auricular acupuncture is a simple treatment that can be administered to patients in groups. The needles are inserted manually, and the points do not need to be electrically stimulated [3], making the procedure virtually painless and easy to use in most clinical settings. Unlike the Traditional Chinese Medicine approach that infers traditional diagnoses and the use of individualized treatment plans (including various combinations of acupuncture, moxibustion and herbs), NADA acupuncture treatment is a standardized detoxification protocol that can be used on any patient. Essentially, this could simplify the use of this protocol as an adjunct therapy.

A standardized acupuncture protocol, like NADA, could also provide an advantage for acupuncture research. One of the issues with acupuncture studies is that they are conducted using only one acupuncture point or a small set of points. This does not reflect what happens in real practice, since a much larger arsenal of points is usually used for any given patient, and the selection of points for any given disease or symptoms varies substantially between patients. NADA is the only acupuncture protocol that uses the same set of points and the same application procedure throughout the treatment for every single patient.

In spite of the widespread use of NADA treatment and the described benefits in controlling withdrawal pain and other craving-related symptoms [6], clinical studies testing the efficacy of NADA protocol produced conflicting results. Clinical trials with small sample sizes have reported effectiveness of NADA treatment in reducing opioid, alcohol and cocaine withdrawal symptoms and cravings [7–16]. In contrast, three large-scale clinical trials did not support the effectiveness of NADA acupuncture in reducing alcohol and cocaine cravings [17–19]. More clinical trials are underway to assess the efficacy of NADA treatment for opioid abuse. A few studies have used animal models to determine the basic mechanisms underlying acupuncture treatment of adverse effects induced by repeated opioid exposure [20–25]. These studies were performed using body acupuncture points, whereas the most common acupuncture treatment for opioid abuse, NADA, has never been tested using animal models.

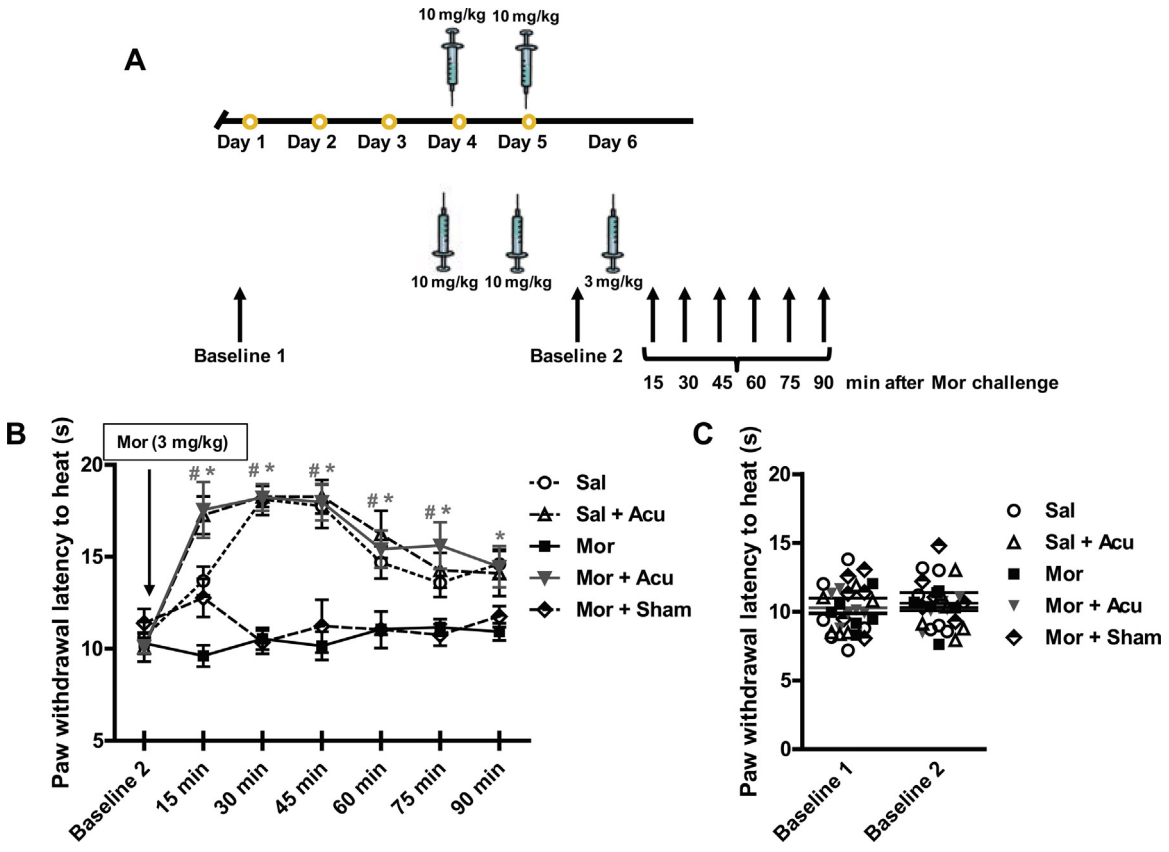

Fig. 2. NADA acupuncture can prevent development of morphine tolerance. (A) Experimental design. (B) Acute morphine challenge (3 mg/kg) produced analgesic effect in saline, but not in morphine treated rats. NADA (but not sham) acupuncture prevented development of morphine tolerance ($n = 6$; $P < 0.0001$; 2-way ANOVA followed by Bonferroni test). Statistical differences were found at the interaction time x group ($n = 6$, $P < 0.0001$, $F = 4.154$). * difference between Mor + Acu and Mor groups, # difference between Mor + Acu and Mor + Sham groups. (C) No changes in the baseline heat sensitivity were observed in any of the treatment groups prior to 3 mg/kg morphine challenge.

Establishing an animal model for studying NADA acupuncture treatment not only would help identifying effective complementary treatment strategies for opioid misuse, but could also provide a basis for the use of the NADA protocol to alleviate adverse effects produced by opioid analgesics in chronic pain treatment. In addition, establishing an animal model for NADA acupuncture treatment could promote future studies of other applications of this therapy, e.g. alcohol and cocaine abuse, stress, etc.

Previous studies, including ours [26–28], have shown that repeated morphine administration in rodents can produce locomotor sensitization and reduce analgesic potency of a challenge dose of morphine, indicating development of morphine tolerance. Here, we show that NADA acupuncture treatment can reduce morphine-induced locomotor sensitization and prevent development of morphine tolerance.

2. Materials and methods

2.1. Subjects

Sprague Dawley 200 g male rats (Harlan) were housed in groups of 2 per cage in a temperature-controlled vivarium on a 12/12 h dark/light cycle with ad libitum access to food and water. Rats were acclimated to the vivarium for at least 2 days before any manipulation. All procedures were approved by the Columbia University Institutional Animal Care and Use Committee in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

2.2. Experimental design and behavioral testing

2.2.1. Locomotor sensitization

Rats were randomly assigned to two treatment groups: 1) Morphine and 2) Saline. Morphine (10 mg/kg) or saline was administered subcutaneously (s.c.) once a day for four days, followed by six days of abstinence. A challenge dose of morphine (5 mg/kg) was administered s.c. on day 11 (one week after the last injection of morphine or saline) (Fig. 1A). This experimental protocol has been previously shown to produce persistent locomotor sensitization in morphine-treated animals, which can be measured as an increase in distance travelled [26,27]. Next, rats in each group were randomly assigned to receive NADA or sham ear acupuncture treatment. NADA protocol was applied using intradermal acupuncture needles to the following ear acupuncture points: shen men, sympathetic, kidney, liver, and lung [3]. Adult rat ear anatomy allows localization of these points (Fig. 1B). Fine 0.22 mm needles were inserted in the right ear under brief isoflurane anesthesia (induced at 2.5% and maintained at 2%, 2L/min O_2) and left in place for 25–30 min (Fig. 1C). In sham acupuncture groups, five needles were inserted in non-acupuncture points along the helix of the ear [1] under the same conditions. Rats received NADA or sham acupuncture treatment once a day (days 13–18). The last acupuncture or sham session was given on day 18 prior to the second challenge of morphine (5 mg/kg s.c.). Locomotor activity was assessed after each injection using locomotor activity chambers equipped with photobeams, as previously reported [26,27].

2.2.2. Analgesic tolerance

Rats were injected s.c. with 10 mg/kg of morphine twice a day for 2 days (total of 4 injections; Fig. 2A). Control group was injected with saline. Acute morphine challenge (3 mg/kg) was administered 12 h after the last 10 mg/kg injection. It has been previously shown that a challenge dose of morphine (3 mg/kg) does not produce an analgesic effect in rats 12 h after the last 10 mg/kg injection due to developed tolerance to morphine [28]. NADA or sham acupuncture treatment was given once a day for 5 days as described above. Hargreaves method [29] was used to assess paw withdrawal latencies [30] to heat stimulation (plantar test). Heat sensitivity was tested: before the first NADA or sham acupuncture treatment (baseline 1); before the administration of morphine challenge (baseline 2); then every 15 min after the morphine challenge (for the total of 90 min).

2.3. Statistical analyses

In the locomotor sensitization experiments a paired *t-test was* used to compare differences within morphine and saline groups (normalized to baseline). A one-way ANOVA test was used to compare differences within and between the groups before and after acupuncture or sham treatment. For tolerance experiments, a two-way ANOVA with repeated measures followed by Bonferroni multiple comparison test was used to compare differences within and between the groups.

3. Results

3.1. NADA acupuncture reduces morphine-induced locomotor sensitization

Previous publications, including our own data [26,27], have shown that repeated morphine administration produces locomotor sensitization in rodents. It has been suggested that locomotor sensitization is a measure of how consistent the use of a drug, such as morphine, produces enhanced craving [31]. We used this model to test whether NADA acupuncture treatment can reduce locomotor sensitization produced by morphine. Morphine (10 mg/kg) administered once a day for four days produced locomotor sensitization on days 2–4 (Fig. 1A; $n = 12$, $p < 0.05$, vs baseline paired *t*-test). Locomotor activity in saline-treated rats remained unchanged. Locomotor sensitization in morphine-treated group was observed one week later, when a smaller challenge dose of morphine (5 mg/kg) was administered after 6 days of abstinence ($n = 12$, $p < 0.05$, vs baseline, paired *t*-test). In saline-treated rats, challenge dose of morphine produced no effect. During the second period of abstinence (6 days) rats in each group were randomly divided into two groups and received either NADA (Fig. 1B) or sham ear acupuncture treatment once a day (days 13–18; Fig. 1C). The last acupuncture or sham session was given on day 18, prior to the second morphine challenge (5 mg/kg). NADA, but not sham (Fig. 1C), ear acupuncture treatment significantly reduced locomotor sensitization produced by morphine ($n = 6$, $p = 0.0022$, $F = 8.280$, one-way ANOVA). Neither NADA, nor sham acupuncture produced any effect in saline-treated groups. Our data demonstrates that NADA auricular acupuncture treatment alleviates morphine-induced locomotor sensitization in a rat model.

3.2. NADA acupuncture can prevent development of morphine tolerance

Next, we tested whether NADA treatment could prevent morphine analgesic tolerance. To induce morphine tolerance, rats were injected with 10 mg/kg of morphine twice a day for 2 days (Fig. 2A) [28]. The control group was injected with saline. NADA or sham acupuncture treatment was given once a day for 5 days starting 3 days before the first injection. Plantar tests (arrows) were performed to assess heat sensitivity. Acute morphine challenge (3 mg/kg) administered 12 h after the last 10 mg/kg injection, as expected, produced analgesic effect in saline, but not in morphine-treated rats (Fig. 2B). NADA acupuncture treatment prevented the development of morphine tolerance ($n = 6$; $P < 0.0001$; 2-way ANOVA followed by Bonferroni test). Rats in sham treatment group exhibited tolerance similar to morphine-treated rats that did not receive any treatment (acupuncture or sham). Statistical differences were found at the interaction time x group ($n = 6$, $P < 0.0001$, $F = 4.154$). Heat sensitivity in Mor + Acu group was significantly different from both Mor and Mor + Sham groups. Interestingly, at 15 min time point paw withdrawal latencies in Mor + Acu group were also statistically different from Sal group, indicating that NADA acupuncture treatment could also shorten the onset of morphine analgesia. No change in the baseline paw withdrawal latencies was observed in any of the treatment groups prior to 3 mg/kg morphine challenge (baseline 1 vs baseline 2; Fig. 2C), indicating that acupuncture treatment by itself did not alter heat sensitivity. These data show that NADA auricular acupuncture treatment not only can reduce morphine-induced locomotor sensitization, but can also prevent development of morphine tolerance and shorten the onset of morphine analgesia.

4. Discussion

Challenges of studying the efficacy of complementary medicine, such as acupuncture, have been described [32]. Using animal models can certainly be advantageous, but also encounters a number of issues. Typically, only one point or a small set of acupuncture points is tested to treat a symptom or a disease, while a much larger number of points is used in clinical practice. Another issue is that in the majority of studies, only the acute effect of point stimulation is investigated. However, healing is a cumulative process, and acute point stimulation can be too weak or too short to produce an effect. Electrical stimulation is normally used in animal acupuncture studies to amplify the effect, while this technique is rarely used in clinical practice and almost exclusively for pain relief. To this end, the NADA acupuncture protocol is unique, since neither the treatment procedure nor the point selection varies between or within the subjects. Five auricular points are manually stimulated once a day and typically a minimum of 8 sessions are required to observe a sustained effect on withdrawal symptoms in humans. Considering the shorter lifespan of rats compared to humans, we used 6 acupuncture sessions during the abstinence period. Our data shows that NADA acupuncture points can be effectively localized on the adult rat's ear and that the effect produced by treatment of these points is similar to the one observed in acupuncture practice. We found that NADA acupuncture treatment in rats could reduce locomotor sensitization produced by repeated morphine administration. Stimulation of placebo (sham) points, selected based on clinical studies [1], did not reduce locomotor activity, indicating specific effects of the point selection. In saline-treated rats acupuncture or sham treatment did not affect locomotor activity.

Repeated opioid administration in the clinical setting is often accompanied by analgesic tolerance, characterized by a continued need for dose escalation to maintain the same level of pain relief. Our data shows that NADA acupuncture treatment can prevent the development of morphine tolerance in an animal model [28]. Preventing opioid dose escalation with NADA acupuncture, consequently, may reduce other opioid-induced adverse reactions such as nausea, vomiting, constipation, sedation, respiratory depression. Furthermore, in our experiments an analgesic effect of morphine in Mor + Acu group was observed 15 min earlier than in Sal group,

indicating that acupuncture treatment could shorten the onset of opioid analgesia. Neither acupuncture nor sham treatment altered paw withdrawal latencies prior to administration of morphine challenge. This indicates that NADA treatment protocol does not affect baseline heat sensitivity. This observation does not exclude the possibility that this protocol may be effective in painful conditions. Future studies are needed to test the effect of NADA treatment in pain models. The main indication for NADA acupuncture treatment, however, has always been detoxification rather than pain relief.

Taken together, our data both establishes and validates a new model for NADA acupuncture studies. It supports the efficacy of NADA treatment in reducing opioid craving observed in clinical studies. In addition, our study provides evidence of the effectiveness of this complementary therapy in preventing morphine tolerance and shortening the onset of morphine analgesia, suggesting a new use of the NADA protocol as an adjunct therapy to opioid analgesics for the treatment of chronic pain.

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