Journal of Neuroscience Methods 201 (2011) 390–398

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Mouse current vocalization threshold measured with a neurospecific nociception assay: The effect of sex, morphine, and isoflurane

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ARTICLE INFO

Article history:
Received 21 April 2011
Received in revised form 3 August 2011
Accepted 4 August 2011

Keywords:
Nociception
Sine-wave
Sex
Opioid
Anesthesia
Morphine

ABSTRACT

Sine-wave electrical stimulation at frequencies 2000, 250, and 5 Hz to respectively evaluate Aβ, Aδ, and C sensory neurons has recently been added to the armamentarium used to evaluate sensory neurons. We developed an automated nociception assay using sine-wave stimulation methodology to determine current vocalization threshold in response to 2000, 250, and 5 Hz and examine the effects of sex, analgesics, and anesthetics in mice. At baseline, males had significantly higher mean current vocalization thresholds compared with female mice at 2000, 250, and 5 Hz ($p \leq 0.019$). By 1 h after intrathecal injections of morphine there were significant increases in current vocalization threshold percent changes from baseline that varied with doses ($p = 0.0001$) and frequency used ($p < 0.0001$). Specifically, with increasing doses of morphine, there were significantly greater increases in current vocalization threshold percent changes from baseline in response to 5 Hz compared with 250 and 2000 Hz stimulation in a significantly ordered pattern: 5 Hz > 250 Hz ($p < 0.0001$) and 250 Hz > 2000 Hz ($p = 0.0002$). Forty-five minutes after exposure, there were no effects of isoflurane on current vocalization thresholds at any frequency. Therefore, our findings suggest that this automated nociception assay using sine-wave stimulation in mice, can be valuable for measurements of the effects of sex, opioids, and anesthetics on the response to electrical stimuli that preferentially stimulate Aβ, Aδ, and C-sensory fibers *in vivo*. This investigation suggests the validation of this assay and supports its use to examine mechanisms of nociception in mice.

1. Introduction

In vivo, the evaluation of specific sensory nerve fibers function [Aβ (pressure), Aδ (localized sharp pain), and C (burning pain) fibers] can be performed with the use of variable rates of noxious radiant heat stimulation as Aδ fibers are activated by high rate and C fibers by low rate of skin heating (Yeomans et al., 1996a,b; Yeomans and Proudfit, 1996). Alternatively, sine-wave electrical stimulation at frequencies of 2000, 250, and 5 Hz to respectively stimulate Aβ, Aδ, and C sensory nerve fibers function can also be used to study specific sensory neurons. The specificity of 5, 250, and 2000 Hz to stimulate C, Aδ, Aβ sensory neurons results from the distinct electrophysiological characteristics (diameter, conduction velocity, and refractory period) of each type of afferent neurons (Katims, 1998; Koga et al., 2005). As such, this methodology has been used in clinical and experimental settings in humans and animals for the diagnosis of neuropathies and for the investigations of pharmacodynamics of analgesics and mechanisms of nociception (Angst et al., 2001; Finkel et al., 2002; Katims, 1998; Katims et al., 1991; Liem et al., 2005; Masson and Boulton, 1991; Matsutomo et al., 2005; Oishi et al., 2002). In rodents and dogs, sine-wave stimulation has also been used to evaluate the effect of analgesics and inflammatory pain models on specific sensory nerve fibers (Kiso et al., 2001; Matsumoto et al., 2006a,b, 2008; Nagakura et al., 2008a,b; Oda et al., 2005; Watabiki et al., 2010). Therefore sine wave stimulation has been shown to be applicable to several species and has added to our ability to evaluate the function of sensory neurons and nocifensive behavior.

We have developed an automated nociception assay using sine-wave stimulation methodology to determine current vocalization threshold in response to 2000, 250, and 5 Hz and thereby examine the function of Aδ, Aβ, and C sensory fibers respectively in mice. In the present investigation, we aimed at validating the assay for the measurements of the effects of sex, opioid analgesia, and anesthetics in mice.

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doi:10.1016/j.jneumeth.2011.08.011

Table 1
Number of animals enrolled per group.

	Baseline sex difference studies				
Strain	C57Bl/6[a]				B6129Sf/J
Male	19				12
Female	53				18
	Morphine effect studies				
Morphine dose	0 μg/mouse	10 μg/mouse	20 μg/mouse	30 μg/mouse	40 μg/mouse
Male	3	4	2	6	4
Female	16	6	8	10	13
	Isoflurane anesthesia study				
Anesthesia	Sham Anesthesia				Isoflurane anesthesia
Male	8				8
Female	8				8

[a] For baseline sex difference studies in C57Bl/6 mice, we included all baseline measurements obtained in animals from the morphine study before any intervention was made. Animals in the morphine study were given only one of the 5 morphine doses. Only C57Bl/6 mice were included in the morphine effect study.

2. Materials and methods

2.1. Mice

After approval from the NIH Clinical Center Animal Care and Use Committee, National Institutes of Health, 87 female and 47 male B6129Sf/J and C57Bl/6 (Jackson Laboratory, Bar Harbor, ME) mice weighing 18–32 g and aged 6–22 weeks were enrolled in this study (Table 1).

2.2. Nociception assay

We designed an assay aiming at eliminating operator variability in the interpretation of nocifensive behavior in mice. As such, in this nociception assay, the delivery of electrical stimuli and the recognition of the nocifensive behavior, here defined as vocalization (audible), are automated. Fig. 1 illustrates the components of the neurospecific nociception assay. Custom hardware and software were designed to control and automate the frequency of electrical stimulation, stimulus delivery/termination, intensity, duration, and duty cycle. The system monitors the progression of experiments, detects mouse vocalizations (here defined as the nocifensive behavior), and records measurements. The electrical stimulus is generated by a neurostimulator (Neurometer, Neurotron, Inc., Baltimore, MD) and is controlled by custom software through a standard RS-232 serial port. A custom built handheld control device is connected through a custom cable to a DAQCard 6533 Digital IO PCMCIA card (National Instruments, Austin, TX) located in the laptop and allows for discontinuation of stimulus. A microphone (AT943-SP, Sound Professionals, Mt Laurel, NJ) is placed on a rubber mount in front of the mouse. The microphone connects to a custom built preamplifier which connects to a DAQ-Card PCMCIA card (National Instruments, Austin, TX). A custom software program controls the devices through drivers provided by the respective companies. The multi-threaded program is written in C++ using object-oriented concepts and uses Microsoft Foundation Classes (MFC) for the graphical interface.

For current vocalization threshold measurements, animals are placed in a mouse holder (Kent Scientific Corporation, Torrington, CT) such that the tail is accessible to the investigator. The mouse holder was modified to minimize mouse chewing and scratching on hard surfaces, which can be a sources of problematic audio noise. Electrodes are applied to the mouse tail using adhesive tape. A grounding (SDE44; Neurotron Inc., Baltimore, MD) electrode is placed at the most proximal end of the tail and a stimulating (ATE1925; Neurotron Inc.) electrode is placed 1 cm distally to the grounding electrode. Cables are snapped on to grounding and stimulating electrodes and connected to the neurostimulator to enable stimuli delivery.

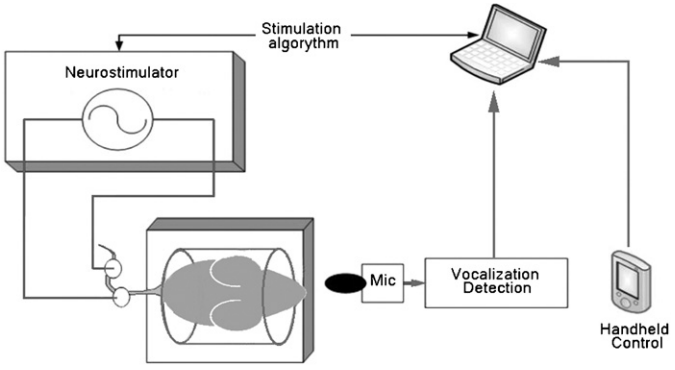

Fig. 1. Components of the nociception assay used to study the effects of sex, opioids and anesthetics in current vocalization threshold to electrical stimuli. The delivery of stimulation and detection of nocifensive behavior recognition is entirely automated.

2.3. Current vocalization threshold

Audible vocalization is the nocifensive behavior end-point used to cease delivery of electrical stimulation. The current vocalization threshold corresponds to the amperage of the electrical stimulus at which vocalization occurs. For ease of data presentation, we defined the unit of measure of current vocalization threshold as units which corresponds to the stimulus intensity (amperage) that yielded nocifensive behavior (vocalization) or the maximum amperage delivered for each frequency multiplied by 100 (Table 2). We conducted pilot studies to examine the characteristics of mouse vocalization in response to the electrical stimulus and found that mouse movements during the experiment could be picked up by the microphone and needed to be distinguished from vocalizations.

Table 2
Characteristics of electrical stimulation algorithms at each frequency delivered to determine current vocalization threshold.[a]

	Frequency		
	5 Hz	250 Hz	2000 Hz
Stimulus characteristics			
Fiber preferentially stimulated	C fiber	Aδ fiber	Aβ fiber
Duration of stimulus at each level	1 s	1 s	1 s
Intensity (minimum–maximum, mA)	0.05–0.5	0.14–0.5	0.4–1.6
Increment intensity (mA)	0.05	0.04	0.1
Respective current vocalization threshold (units)	5–50	14–50	40–160

[a] One current vocalization threshold unit is equal to 100 mili Amperes (mA).