



Basic neuroscience

Use of von Frey filaments to assess nociceptive sensitization in the hornworm, *Manduca sexta*



Marissa Zubia McMackin^a, Matthew R. Lewin^b, Dennis R. Tabuena^a, F. Eric Arreola^a, Christopher Moffatt^a, Megumi Fuse^{a,*}

^a Department of Biology, San Francisco State University, 1600 Holloway Ave, San Francisco, CA 94132, USA

^b Institute for Biodiversity Science and Sustainability Director Center for Exploration and Travel Health, California Academy of Sciences, 55 Music Concourse Dr., Golden Gate Park, San Francisco, CA 94118, USA

HIGHLIGHTS

- Von Frey filaments were used to quantify the defensive strike response in *M. sexta*.
- The first response, up-and-down and SUDO methods were assessed and compared.
- All 3 methods measured changes in threshold sensitivity after a noxious pinch.
- Nociceptive sensitization after the pinch was determined to last 19 h.

ARTICLE INFO

Article history:

Received 26 August 2014
Received in revised form
16 September 2015
Accepted 19 September 2015
Available online 9 October 2015

Keywords:

Defensive strike response
Larva
Von Frey filaments
SUDO method
Up-and-down method
Insect

ABSTRACT

Background: The hornworm *Manduca sexta* exhibits a defensive strike to noxious assaults, a response that is robust and is easily observed by experimenters. Von Frey filaments and methods typical for studying nociception in other animals were used to assess the strike response in *M. sexta*.

New methods: A series of von Frey filaments was applied to the body wall in ascending order and the data generated were used to determine the strike threshold by (i) the up-and-down method, (ii) the first response method, and (iii) the simplified up-and-down order method (SUDO). The effect of a noxious pinch on strike threshold was assessed.

Comparison with existing methods: To our knowledge none of these methods has been used on *M. sexta* previously, making the use of the up-and-down and SUDO methods the first in an invertebrate. The use of the first response method has been used in other invertebrates, and the method appears equally suited to *M. sexta*.

Results: All three methods were successful in monitoring the threshold sensitivity to touch, which was lowered (sensitized) by tissue damage induced with a pinch. Sensitization lasted 19 h.

Conclusions: All three methods of assessing nociception were successfully applied to quantify the defensive strike response in *M. sexta*, although the SUDO method required empirical assessment of which filament to start the test sequence with. The results revealed both short- and long-term sensitization. These methods should prove to be useful for quantifying sensitization in *M. sexta*.

Published by Elsevier B.V.

Abbreviations: L5, fifth larval stage; F₅₀, force threshold for 50% chance to strike; SUDO, simplified up-and-down order method; Filament #, filament number.

* Corresponding author. Tel.: +1 415 405 0728; fax: +1 415 338 1130.

E-mail addresses:

mzmcmackin@gmail.com (M.Z. McMackin), mlewin@calacademy.org (M.R. Lewin), dennis.r.tabuena2@gmail.com (D.R. Tabuena), arreola.eric85@gmail.com (F.E. Arreola), moffatt@sfsu.edu (C. Moffatt), fuse@sfsu.edu (M. Fuse).

<http://dx.doi.org/10.1016/j.jneumeth.2015.09.015>

0165-0270/Published by Elsevier B.V.

1. Introduction

Nociception, which includes the perception and processing of noxious stimuli, is a response to potential or actual tissue damage. The nervous system may then be sensitized in response to noxious stimuli, as a form of non-associative learning that may last days to months (e.g., Castellucci et al., 1986; Woolf and Walters, 1991). This presumably serves to heighten the ability of individuals to respond to and protect themselves from potentially harmful events and protect injured areas during healing. In humans, such noxious stimuli

manifest in the subjective experience of pain (reviewed by Walters, 1994; Rahn et al., 2013), and the result of abnormally persistent nociceptive sensitization includes conditions such as allodynia (such as that experienced on the surface of a scar long after an operation or wound), where normally innocuous stimuli are interpreted as noxious or painful), or hyperalgesia, where painful stimuli are perceived as more painful than normal. These neuropathic conditions can occur following a single insult or after repeated injury, chronic inflammation and in some genetic conditions (Binder et al., 2011).

In humans, the severity of pain a person is experiencing is typically ascertained through verbal or other subjective ratings of discomfort, although quantitative sensory testing (QST) may become the standard clinically and in clinical research (reviewed by Backonja et al., 2009; Cruz-Almeida and Fillingim, 2014). In this case, von Frey filaments, which provide a calibrated force to a portion of the subject's body, are used to test the mechanical sensitivity. In fact, the use of these filaments has been exploited for the precise measurement of mechanosensitivity in most vertebrates in pain research. In models such as rats, defensive behaviors are monitored by a number of different assays. For instance, the tail flick, hind paw withdrawal and jump-flinch are all responses to noxious stimuli that may be sensitized after formalin injection, high-intensity light beams, hot plates or after spinal nerve ligation surgery (e.g., Juskiewicz-Donsbach and Levy, 1962; Bonnet and Peterson, 1975; Pitcher et al., 1999; Kim et al., 2014; reviewed by Bannon, 2001; Bannon and Malmberg, 2007; Gregory et al., 2013). These responses are quantified by assessing either the latency to respond to the noxious stimulus or the intensity of the stimulus causing a response (the threshold response).

For years, most threshold response measurements using von Frey filaments recorded the “first response” to stimulation, with an ascending series of increasingly stiff filaments (e.g., Illich and Walters, 1997; Alupay et al., 2014). The “up-and-down” method, that was originally designed to estimate the stability of explosives, was first used in animal models to estimate lethal doses of drugs (Dixon and Mood, 1948; Dixon, 1965) and has since become the standard for assessing vertebrate nociception (Crocker and Russell, 1984; Chaplan et al., 1994). This method starts with a mid-range filament, and increases or decreases linearly, depending on the response of the animal. A defensive response results in a lower filament force being used next, while lack of a response dictates that a higher strength filament be used for the next test. This is repeated a number of times, and a table of probability thresholds was developed in rodents, depending on the sequence of responses and a maximum likelihood model (Dixon and Mood, 1948; Dixon, 1965; Chaplan et al., 1994). More recently, a simplified up-down order (“SUDO”) method was developed, where only five stimuli are required to determine nociceptive threshold, without the need for a look-up table. In this method, each animal receives the same number of touches from von Frey filaments. If the last touch by the filament yields a response, a set value is added to the filament force, whereas if no response is noted, a set value is subtracted from that filament force (Bonin et al., 2014; see also methods Section 2.5). This method, however, requires empirical determination of a suitable starting force.

A number of invertebrate nociceptive models exist, the most well studied being *Aplysia californica* (reviewed by Walters and Moroz, 2009). The breadth of invertebrate studies has provided evidence of the relationship of invertebrate sensitization mechanisms to mammalian pain and memory mechanisms (e.g., Walters and Moroz, 2009; Crook and Walters, 2011; Crook et al., 2013). In many of these studies, the “first response” method of assessment was used to show nociceptive sensitization of behavior (e.g., Lewin, 2003; Alupay et al., 2014) and of mechanosensory neurons (Illich and Walters, 1997). Short and long-term sensitization of the siphon

and tail withdrawal in *A. californica* paired with electrophysiology led to a fundamental understanding of the enhancement of synaptic transmission between sensory and motor neurons, which were important building blocks in the evolution of learning and memory (e.g., Carew et al., 1983; Walters, 1987; Schacher et al., 1988; Lewin and Walters, 1999). In *Caenorhabditis elegans*, thermal avoidance behavior was characterized through video monitoring of whole worm movements (e.g., Wittenburg and Baumeister, 1999). In insects, *Drosophila* was monitored visually for thermal avoidance behaviors after a variety of genetic manipulations (Tracey et al., 2003; Neely et al., 2010; Babcock et al., 2011; reviewed by Kim et al., 2011).

The hornworm, *Manduca sexta*, is another possible model organism for such research. It has conspicuous and reproducible defensive behaviors that include proleg withdrawal (Wiel and Weeks, 1996) and defensive strike and thrashing responses (Walters et al., 2001) to predators such as birds. The change in the number of defensive strikes for a set stimulus was the original method used for assessing nociceptive sensitization (Walters et al., 2001). In this case, the behavioral change associated with sensitization was recorded for less than an hour, whereas sensitization has been noted for much longer periods in many other invertebrates (e.g., Gasull et al., 2005). Moreover, this assay depended on an all-or-none response, and subtle changes in nociceptive sensitivity were not noted. Finally, a sequence of 16 applications of the von Frey filaments was required, leaving the possibility of habituation as a confounding factor.

Developing an invertebrate model using the first response, up-and-down and SUDO methods should prove important in understanding the conservation of pain signaling in animals, but may also alleviate ethical issues associated with testing vertebrates. The conserved nature of pain receptors such as the TRP receptors (e.g., Kim et al., 2012; Saito et al., 2012; Sardar et al., 2012; Palovcak et al., 2015), and associated signal transduction machinery (e.g., Lin et al., 1997; Lewin and Walters, 1999; Nikitin and Kozyrev, 2000) suggest that such a model will be invaluable in this field of research. Using an organism such as *M. sexta* is cheap and currently unregulated, allowing rapid and large scale testing of signaling mechanisms and pharmaceuticals. The goal of our research was to adapt and test the first response, up-and-down and SUDO methods to quantify the defensive strike response in *M. sexta*. To our knowledge, the use of the up-and-down and SUDO methods has not yet been incorporated into the quantification of any invertebrate defensive responses. *M. sexta* may thus provide a valuable tool for studying the signal transduction mechanisms inducing sensitivity after injuries leading to hyperalgesia or allodynia as well as the role of long-term memory in providing nocifensive behaviors in animals.

2. Materials and methods

2.1. Animals

M. sexta L. were raised under a 17:7 h photoperiod with a thermoperiod of 27 °C:25 °C as described previously (Fuse and Truman, 2002). Free feeding was allowed on an artificial diet (MP Biomedical Irvine CA USA: based on Bell and Joachim, 1976). Animals were individually housed in small plastic cups with lids. Pharate fifth instar larvae were staged using external morphological markers (Truman et al., 1980; Copenhaver and Truman, 1982). Each animal was observed and timed for ecdysis and allowed to feed ad libitum and rest in the incubator for 24 h. Larvae were tested and provided stimuli 24 h after ecdysis to the fifth larval stage (L5) and then retested 1 and 19 h after the presentation of the noxious stimulus.

Download English Version:

<https://daneshyari.com/en/article/6268096>

Download Persian Version:

<https://daneshyari.com/article/6268096>

[Daneshyari.com](https://daneshyari.com)