Neuroscience and Biobehavioral Reviews 51 (2015) 223–242

Contents lists available at ScienceDirect

Neuroscience and Biobehavioral Reviews

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

Review

Comparison of operant escape and reflex tests of nociceptive sensitivity

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ARTICLE INFO

Article history:
Received 28 September 2014
Received in revised form 17 January 2015
Accepted 27 January 2015
Available online 3 February 2015

Keywords:
Flexion/withdrawal reflex
Operant escape
Lick/guard reflexes
Chronic constriction injury
Spinal nerve ligation
Spinal cord injury
Aging
Sex differences
Morphine
Psychological stress
Neuropathic pain

ABSTRACT

Testing of reflexes such as flexion/withdrawal or licking/guarding is well established as the standard for evaluating nociceptive sensitivity and its modulation in preclinical investigations of laboratory animals. Concerns about this approach have been dismissed for practical reasons – reflex testing requires no training of the animals; it is simple to instrument; and responses are characterized by observers as latencies or thresholds for evocation. In order to evaluate this method, the present review summarizes a series of experiments in which reflex and operant escape responding are compared in normal animals and following surgical models of neuropathic pain or pharmacological intervention for pain. Particular attention is paid to relationships between reflex and escape responding and information on the pain sensitivity of normal human subjects or patients with pain. Numerous disparities between results for reflex and operant escape measures are described, but the results of operant testing are consistent with evidence from humans. Objective reasons are given for experimenters to choose between these and other methods of evaluating the nociceptive sensitivity of laboratory animals.

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http://dx.doi.org/10.1016/j.neubiorev.2015.01.022
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Previously expressed concerns about the prevalent methods of testing nociceptive sensitivity of laboratory animal subjects (Vierck, 1991, 2005, 2006a; Vierck et al., 1989, 2008b) have not stemmed the tide of investigations intending to study pain by monitoring simple reflex responses to nociceptive stimulation. Consistent use of reflex tests persists on the bases of simplicity (e.g., no need for pretraining) and an implicit assumption that limb withdrawal represents a conscious attempt to escape from continuance of a sensation of pain. However, to conclude that a sensation of pain precedes and motivates reflex withdrawal from a stimulus, it is necessary to determine that reflex responses have characteristics that match consciously directed escape.

1. Comparison of reflex and operant escape responses to nociceptive stimulation of normal monkeys, rats and humans

For stimuli of sufficient intensity to elicit reflexes, response latencies distinguish flexion/withdrawal reflexes from operant escape responses. In studies of *Macaca speciosa* monkeys and Long-Evans rats, electrical stimulation has been delivered to one hindlimb while simultaneously recording flexion/withdrawal of the stimulated limb and operant escape. Manual lever pulls (monkeys) or bar press responses (rats) with either hand terminate stimulation of the leg that responds with flexion/withdrawal (Cooper and Vierck, 1986; Greenspan et al., 1986; Vierck et al., 1990, 1995; Vierck and Light, 1999). Reflex responses to brief electrical stimulation (50 ms duration) occur within 100 ms (e.g., see Fig. 4). The minimum reaction time for sensory perception and then a conscious response to somatosensory stimulation is 250 ms (Fendrich et al., 2004). Therefore, the sequence of neural events necessary for the animal to perceive that flexion/withdrawal has escaped painful electrical stimulation is not present. For reflex testing with electrocutaneous stimulation, the sensation occurs during or after the reflex response, which does not terminate pain. The pain is inescapable from the animals' perspective. In contrast, an escape response occurs following onset of a painful sensation, which is extinguished only after and obviously by occurrence of the operant response.

There are important differences between reflex and operant escape responses to stimuli which vary in intensity. Hindlimb flexion is generally regarded as a nociceptive reflex, but it occurs for low intensities of electrical stimulation (below 0.4 mA/mm^2) that are not escaped. The low intensities that elicit reflex responses but not escape are above the threshold for detection of electrical stimulation by monkeys and human subjects, but they are below the pain threshold of human subjects (Ellrich and Treede, 1998; Greenspan et al., 1986; Spaich et al., 2004; Vierck et al., 1983b). The sensation produced by low intensity electrical stimulation of non-nociceptive afferents is described as a tap, or tingling, or occasionally as itch, depending upon the site and the parameters of stimulation (Vierck et al., 1995). Surely, no one would suggest that central encoding of cutaneous sensations such as these can be understood by studying flexion/withdrawal responses to non-nociceptive stimulation. Similarly, visual or auditory reflexes are not utilized to investigate mechanisms of sensory perception in these modalities. Assignment of sensory perception to characteristics of a reflex response is unique to the study of pain.

In the experiments with monkeys, the frequency of responding, the force of hindlimb flexion and the force of bar-pull escape responses to electrocutaneous stimulation have been recorded, in addition to response latencies. For stimulus intensities suprathreshold for escape (0.6, 1.1 and 2.2 mA/mm^2) the speed (1/latency) and force of escape responses increase linearly as the percentage of escape responses progresses from 50% to 100%. In contrast, each of these intensities consistently elicits hindlimb flexion (100%), and the force and speed of the reflexes is almost constant across the range of painful stimulus intensities. Thus, the frequency and amplitude of flexion/withdrawal of a hindlimb in response to brief electrical stimulation are not related to the frequency, speed or vigor of escape. In contrast, the measures of escape responding are proportionate to the presence of pain and its intensity, as judged by human observers (Vierck et al., 1983a; Vierck and Cooper, 1984).

Nociceptive electrical stimulation provides abrupt input that is ideally suited for elicitation of flexion/withdrawal responses. Low to moderate intensities of electrical stimulation that elicit pain preferentially activate myelinated (A-beta and A-delta) afferents, with fast transmission of high discharge rates to the spinal cord. However, clinical pain often is associated with activation of unmyelinated (C) nociceptors that slowly conduct low rates of sustained discharge (Cooper et al., 1986; Vierck et al., 1984). Thermal stimulation can preferentially activate A-delta or C nociceptors, depending upon the temperature and the duration and frequency of stimulation. Also, C-and A-fiber activation can be mutually inhibitory at cerebral sites of nociceptive processing (Vierck et al., 2013c), with dominance of C or A-delta pain, depending upon the stimulus and its duration. Thus, it is instructive to compare reflex and operant escape responses to a range of cold and hot thermal stimuli that activate different combinations of myelinated and unmyelinated nociceptors.

High temperatures that elicit cold pain predominantly activate A-delta nociceptors; low cold temperatures also stimulate C