Laboratory Tools and their Relevance for Assessing Patients with Neuropathic Pain

Andrea Truini, MD
Department of Neurology and Psychiatry, Sapienza University, Rome, Italy

Educational Objectives

1. Describe neurophysiological techniques for assessing peripheral and central somatosensory pathways in patients with neuropathic pain.

2. Describe the use of skin biopsy for assessing somatosensory pathways in patients with neuropathic pain.

Introduction

Research has devised various techniques for investigating nociceptive and non-nociceptive somatosensory pathways in patients with neuropathic pain. The most widely accepted tools in use today are neurophysiological techniques and skin biopsy.

Neurophysiological Techniques

The standard neurophysiological techniques such as nerve conduction studies, trigeminal reflexes, and somatosensory evoked potentials are mediated by large non-nociceptive afferent fibers (Aβ fibers) and are widely used for assessing peripheral and central nervous system diseases [6]. Because most clinical and experimental studies show that neuropathic pain is mainly related to nociceptive pathway damage, current knowledge postulates that the neurophysiological assessment of large non-nociceptive afferent fibers does not contribute to the diagnosis of neuropathic pain. However, these standard techniques are still useful to demonstrate, locate, and quantify damage along the peripheral and central somatosensory pathways [6,9]; furthermore, some recent studies have suggested that some specific types of neuropathic pain are specifically associated with Aβ-fiber damage. Recent neurophysiological studies have shown that in patients with peripheral and central nervous system disease, paroxysmal electric-shock-like sensations are associated with abnormalities of neurophysiological responses mediated by non-nociceptive Aβ fibers [17,18,21].

Guidelines on neuropathic pain assessment by the IASP Neuropathic Pain Special Interest Group (NeuPSIG) [9] indicate that laser-evoked potentials (LEPs) are the best-studied and most reliable neurophysiological technique for assessing nociceptive pathway function. Laser-generated radiant heat pulses selectively excite free nerve endings in the superficial skin layers and activate Aδ and C nociceptors [14]. Although laser stimuli activate both Aδ and C fibers, scalp potentials related to C-fiber activation (C-LEPs) can be obtained only with dedicated techniques that have not yet been standardized for clinical application [6]. Hence, the commonly studied LEPs are those related to Aδ-fiber activation. LEPs consist of a lateralized component (N1), generated in the S2 area and in the insular cortex.
### Methods supporting clinical examination in patients with neuropathic pain

**Left column: stimulation. Right column: recording.**

- **Blink reflex (Aβ fibres)**
  - R1
  - R2

- **Nerve conduction study (Aβ fibres)**
  - Median SNAP

- **Somatosensory evoked potentials (Aβ fibres)**
  - N9
  - N20
  - P25

- **Laser evoked potentials (Aδ fibres)**
  - N1
  - N2
  - P2

- **Skin biopsy (intraepidermal nerve fibres)**

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*Fig. 1.* Methods supporting clinical examination in patients with neuropathic pain. Left column: stimulation. Right column: recording. The blink reflex (recorded from the orbicularis oculi muscle), nerve conduction study, and somatosensory evoked potential and laser-evoked potential (both recorded from the scalp) are neurophysiological methods that assess function of large or small fibers. Skin biopsy allows the count of epidermal nerve fibers (ENFs), thus measuring the degree of small-fiber loss (modified from [20]).
bilateral, and a vertex potential consisting of an N2–P2 complex [8]. Whereas the N2-LEP component probably reflects neuronal activity in the insular and possibly the anterior cingulate cortex, the P2-LEP originates from the anterior cingulate cortex alone [8]. Currently, solid-state lasers (mainly Thulium or Neodinium-based lasers) are the most widely used laser stimulators [16, 23]. They use short wavelengths (1.2 nm), ensuring a steep temperature rise and thus synchronizing afferent volleys, which enhances the amplitude of cortical responses [23]. Recommendations for the clinical use of somatosensory-evoked potentials of the International Federation of Clinical Neurophysiology [2] suggest using a minimum of four recording electrodes: two midline sites (Fz–Cz) referred to the nose (or earlobes) to record the vertex N2 and P2 components, one contralateral temporal electrode referred to the midline (Fz or better Fpz) to record the early N1 component, and one electrooculogram site to detect and eliminate ocular artefacts.

The LEP amplitude is higher after face stimulation than after hand and foot stimulation [19]. All studies dealing with trigeminal LEPs have reported that facial stimulations yield lower-threshold and higher-amplitude LEPs. Because of a high receptor density in the facial skin and the very short conduction distance, LEP recordings after trigeminal stimulation are easier and quicker to obtain than those after stimulation of the limb extremities. Many studies also show that LEP amplitude negatively correlates with age [3]. Studies addressing the influence of age on LEPs have consistently found an age-related decrease in LEP amplitude, possibly because of a mild neuronal loss or dysfunction in the peripheral nerves or in the brain with advancing age, as happens for other evoked potentials [3, 19]. The amplitude of the N2–P2 complex is also influenced by attention, as shown by experimental findings that the N2–P2 complex is enhanced by attention to stimuli (counting), in comparison with distraction from the stimuli [23].

In diseases associated with nociceptive pathway damage, LEPs can be absent, reduced in amplitude, or delayed in latency [23]. Previous studies have shown that in patients with neuropathic pain related to peripheral and central nervous system diseases (postherpetic neuralgia, carpal tunnel syndrome, polyneuropathy, or multiple sclerosis), the severity of ongoing burning pain is inversely related to LEP amplitude [7, 16–18, 21]. This relationship—although only an indirect finding in some instances—indicates that ongoing burning pain is strongly associated with damage to the nociceptive system [20].

Among pain-related evoked potentials, contact heat evoked potentials (CHEPs) are also commonly used to investigate patients with neuropathic pain, and several studies have indicated that CHEPs selectively investigate nociceptive pathway function. However, this technique has been applied only in small series or case reports, and thus further studies are still needed to assess the clinical usefulness of CHEPs in patients with neuropathic pain [6, 20]. Concentric electrodes have also recently been introduced to measure pain-related evoked potentials (PREPs) in order to assess nociceptive pathways in patients. However, systematic studies suggest that concentric electrodes might also activate non-nociceptive Aβ fibers. In at least one clinical study, PREPs failed to detect nociceptive pathway lesions that were readily identified by LEPs in patients with Wallenberg syndrome and syringomyelia. Hence, the clinical usefulness of PREPs for assessing nociceptive pathway function is still debated [6, 20].

Microneurography can be used to record individual action potentials from single fibers, thereby quantifying spontaneous activity from nociceptive fibers. Although this technique is not part of the clinical routine, microneurographic studies for research purposes in selected groups of patients with various neuropathic pain conditions have helped clarify the contribution of abnormal spontaneous activity from nociceptive fibers to neuropathic pain symptoms [10].

**Skin Biopsy**

Skin biopsy is a reliable and minimally invasive tool for investigation of nociceptive fibers in human epidermis and dermis [13]. Researchers have used this technique for assessing epidermal nerve fibers (ENFs) qualitatively and quantitatively. Skin biopsy can be performed at any site of the body, with a disposable punch, using a sterile technique, and under local anesthesia. A 3-mm diameter punch is commonly used, with no need for sutures. For diagnostic purposes in peripheral neuropathy, a skin biopsy is commonly taken at a distal site on the leg (10 cm above the lateral malleolus) and a further biopsy is taken at a proximal site on the thigh (20 cm below the iliac spine); thus, a proximal site and a distal site can be assessed if a length-dependent process is suspected [13]. Punch biopsy produces a sample of skin
that includes the epidermis and the superficial dermis. Immunostaining of 50-mm thick sections labels the different structures (e.g., nerve fibers, sweat glands, blood vessels, and resident or infiltrating cells) [11]. The most commonly used marker for nerve fibers are antibodies against protein gene product (PGP) 9.5, a form of ubiquitin carboxyl-terminal hydrolase. PGP 9.5 is widely distributed in the peripheral nervous system and is a nonspecific panaxonal marker [11]. Antibodies against specific myelin components are used to investigate unmyelinated and myelinated cutaneous nerve fibers. Autonomic fibers innervating sweat glands and blood vessels can be immunostained with antibodies against neuropeptides such as vasoactive intestinal peptide, substance P, or calcitonin gene-related peptide [3].

The two most commonly used immunostaining methods for investigating cutaneous innervation are bright-field immunohistochemistry and indirect immunofluorescence with or without confocal microscopy. Only the bright-field microscopy method has been used to establish normative reference ranges and diagnostic performance. However, because immunohistochemical techniques have no influence on the diagnostic efficiency of skin biopsy, the European Federation of Neurological Societies guidelines on the use of skin biopsy in the diagnosis of peripheral neuropathy indicate that immunohistochemistry with bright-field microscopy or immunofluorescence is equivalent in assessing ENF density [11].

Many investigators have used skin biopsy to investigate ENFs in various peripheral nerve diseases, such as diabetic neuropathy, infectious and inflammatory neuropathies, and neuropathies associated with systemic diseases [5,11,13]. In all studies, ENF density was significantly lower in patients with neuropathy than in controls. Skin biopsy allows the neurologist to investigate small nerve fibers with different functions (e.g., somatic unmyelinated fibers within the epidermis, and the autonomic fibers innervating sweat glands and arrector pili muscles) [3,11]. Skin biopsy can help in assessing the primary site of nerve damage. In length-dependent axonal polyneuropathies, such as those occurring in diabetes, ENF density is typically reduced in the distal rather than the proximal leg, reflecting the dying-back degenerative process. Conversely, in sensory neuropathies caused by primary degeneration of dorsal root ganglia neurons, such as those associated with neoplasms or Sjögren’s syndrome, the histological findings from skin biopsy may disclose a pattern of non-length-dependent skin denervation.

Although skin biopsy selectively assesses ENF density and reliably diagnoses small-fiber neuropathy, published studies report contradictory results on the relationship between skin biopsy data and pain [1,4,12,22]. Whereas some studies have reported a possible relationship between ENF density and pain, most investigators found none. A recent skin biopsy study in 139 patients with peripheral neuropathy analyzed the relationship between ENF density and the different types of neuropathic pain [15]. This study showed that ENF density did not differ between patients with and without ongoing burning pain, thus suggesting that this type of pain does not merely reflect axonal loss. Conversely, patients with provoked pain, such as dynamic mechanical allodynia, have a higher ENF density than patients without these types of pain, thus raising the possibility that in some patients, provoked pains arise from spared and sensitized nociceptive nerve terminals [20].

**Conclusion**

Neurophysiological techniques and skin biopsy provide reliable information on somatosensory pathways, thus helping in the clinical diagnosis and management of patients with neuropathic pain.

**References**


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Correspondence to: Andrea Truini, MD, Department of Neurology and Psychiatry, University Sapienza, Viale dell’Università 30, Rome 00198, Italy. Email: andrea.truini@uniroma1.it.