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Regular Article

Mirror-image pain after nerve reconstruction in rats is related to enhanced density of epidermal peptidergic nerve fibers

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ABSTRACT

Mirror-image pain is a phenomenon in which unprovoked pain is detected on the uninjured contralateral side after unilateral nerve injury. Although it has been implicated that enhanced production of nerve growth factor (NGF) in the contralateral dorsal root ganglion is important in the development of mirror-image pain, it is not known if this is related to enhanced expression of nociceptive fibers in the contralateral skin.

Mechanical and thermal sensitivity in the contralateral hind paw was measured at four different time points (5, 10, 20 and 30 weeks) after transection and immediate end-to-end reconstruction of the sciatic nerve in rats. These findings were compared to the density of epidermal (peptidergic and non-peptidergic) nerve fibers on the contralateral hind paw.

Mechanical hypersensitivity of the contralateral hind paw was observed at 10 weeks PO, a time point in which both subgroups of epidermal nerve fibers reached control values. Thermal hypersensitivity was observed with simultaneous increase in the density of epidermal peptidergic nerve fibers of the contralateral hind paw at 20 weeks PO. Both thermal sensitivity and the density of epidermal nerve fibers returned to control values 30 weeks PO.

We conclude that changes in skin innervation and sensitivity are present on the uninjured corresponding side in a transient pain model. Therefore, the contralateral side cannot serve as control. Moreover, the current study confirms the involvement of the peripheral nervous system in the development of mirror-image pain.

Introduction

Contralateral pain, also known as mirror-image pain, is a mysterious phenomenon in which patients with trauma or inflammation of the ipsilateral hind paw also feel pain at the non-injured contralateral side. Mirror-image pain after peripheral nerve injury has been suggested to evoke a time-dependent reorganization of both the central and peripheral nervous system (Obata et al., 2010). In literature, central sensitization and spinal glia activation are still playing the leading role in current hypotheses of the potential underlying mechanism of mirror-image pain (Jaggi and Singh, 2011; Milligan et al., 2003; Obata et al., 2010; Racz et al., 2008). Although these studies favor a mechanism that involves the central nervous system in the development of mirror-image pain (Hatashita et al., 2008), a recent study has suggested an important role for the peripheral nervous system (Cheng et al., 2014). These authors demonstrated that after peripheral nerve injury, tumor necrosis factor a (TNF-a) produced in the dorsal root ganglion (DRG) on the injured side diffuses via cerebrospinal fluid to the contralateral DRG where it activates satellite glia to produce nerve growth factor (NGF), which was supported by others (Carcamo, 2014; Jancalek et al., 2010a). It is one of the possible signaling mechanisms, however, we cannot exclude neuronal and/or glial signaling pathways.

NGF is known to promote outgrowth and regeneration of the peptidergic subgroup of sensory nerve fibers (Forrest and Keast, 2008). While these peptidergic nerve fibers contain calcitonin gene-related peptide (CGRP), the remaining epidermal non-peptidergic sensory nerve fibers express the plant lectin isolectin B4 (IB4) and are promoted by glial cell line-derived neurotrophic factor (GDNF) (Stucky and Lewin, 1999).

Although mirror-image pain has been described as a persistent pain syndrome detected by nerve fibers in the skin (Seltzer et al., 1990), no data is available about contralateral changes in the two subgroups of epidermal sensory nerve fibers. The importance of distinction between subgroups of epidermal nerve fibers was demonstrated in an experimental peripheral nerve injury model in which the sciatic nerve was ligated and immediate end-to-end reconstructed was performed (Kambiz et al., 2014b, 2015). This reconstruction is shown to result in

Abbreviations: CGRP, calcitonin gene-related peptide; DRG, dorsal root ganglion; GDNF, glial cell line-derived neurotrophic factor; IB4, isolectin B4; NGF, nerve growth factor; P2X3, purinogenic 2X3; PGP9.5, protein gene product 9.5; PO, postoperation; TNF-a, tumor necrosis factor-a; TRP, transient receptor potential.

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transient ipsilateral pain that was related to increased density of epidermal peptidergic nerve fibers. However, as yet, it is unknown if this model also induces mirror-image pain. Therefore, in the current study, mirror-image pain is examined by determining the thermal and mechanical sensitivity in the contralateral hind paw after unilateral end-to-end reconstruction. Moreover, the behavioral results are compared to the density of epidermal peptidergic and non-peptidergic nerve fibers to provide further insight of the correlation between mirror-image pain and subtypes of sensory epidermal nerve fibers.

Materials and methods

Animals

Experiments were performed on adult female Lewis rats ($n = 30$), weighing between 180 and 200 g. Animals were pair-housed in hooded cages at room temperature on a 12-hour light/dark schedule, and were given food ad libitum. All experiments were approved by the Dutch Ethical Committee on Animal Welfare (DEC) according to the European guidelines for the care and use of laboratory animals (Council Directive 86/6009/EEC).

Surgical procedure

Under isoflurane (3%) anesthesia, the left sciatic nerve of all 30 animals was exposed through a gluteal muscle-splitting approach using a surgical microscope (Zeiss OP-MI 6-SD; Carl Zeiss, Goettingen, Germany) as described previously (Kambiz et al., 2014b, 2015). Subsequently, in 24 animals the sciatic nerve was transected by cutting the nerve with a sharp scissor proximal to its trifurcation. Transection was immediately followed by an epineural end-to-end reconstruction using 6 10/0 Ethilon sutures. The remaining 6 animals served as control in which the sciatic nerve was only exposed without transection. The split muscle and skin of all animals were closed using Vicryl Rapide sutures. In all cases, postoperative analgesia was provided by subcutaneous administration of buprenorphine (0.05–0.1 mg/kg; Temgesic). Animals were monitored daily for signs of stress or discomfort.

Experimental groups

All end-to-end reconstructed animals were randomly divided into four groups consisting of six rats each. Different survival times (5, 10, 20 and 30 weeks after reconstruction) were allowed for each group. The remaining group consisted of the control animals (sham-operated) and was allowed a survival time of 5 weeks. The effect of lesion and its recovery was determined by various behavioral and physiological experiments and immunohistochemistry on the glabrous skin of the contralateral hind paw.

All functional and histological data are obtained from the contralateral hind paw after unilateral end-to-end reconstruction of the sciatic nerve or sham-operation.

Pin-prick test

The pin-prick test was used to estimate the advancement of the area demonstrating recovery of nociception (Kovacic et al., 2003; Nixon et al., 1984). The lateral and medial skin of the hind paw was pinched with a fine forceps starting distally at the toes and ascending up towards the ankle.

Von Frey test

The von Frey test was performed to determine the mechanical sensitivity threshold in the medial and lateral regions of the hind paw using a set of von Frey hairs ranging from 2 g to 300 g in a set of 16 filament steps. The rat was placed in a chamber with a mesh metal floor and each filament was indented for 4 s on the glabrous skin of the hind paw. The stimulus was repeated five times, and was scored positive when three paw flicks (the animal's reflex withdrawal response) were observed. The thresholds for withdrawal responses were compared between the hind paw of the end-to-end reconstructed animals and the hind paw of the sham operated animals. The hind paw was considered to be mechanical hypersensitive when a significant decrease of withdrawal threshold as response to von Frey monofilaments was noted in comparison to control.

Hot and cold plate

The hot and cold plate test was used to assess thermal hypersensitivity in the hind paw. All animals were placed in a light open cage with clear walls and a surface temperature of 5 °C (cold plate) or 50 °C (hot plate). The time until hind paw withdrawal or licking was observed. Significant differences between the end-to-end nerve reconstructed and the control group served as an indication of thermal hypersensitivity.

Electromyography (EMG)

Innervation of motor axons was evaluated by recording evoked compound muscle action potentials (CMAPs) of the gastrocnemius muscles (Werdin et al., 2009). Under general anesthesia, the sciatic nerve was exposed through a gluteal muscle-splitting approach using a surgical microscope (Zeiss OP-MI 6-SD; Carl Zeiss, Goettingen, Germany). A monopolar needle stimulation electrode was placed proximal from the trifurcation of the sciatic nerve to stimulate the nerve (Nijhuis et al., 2011). For recordings, an active electrode was positioned over the midpoint of the medial gastrocnemius muscle with the

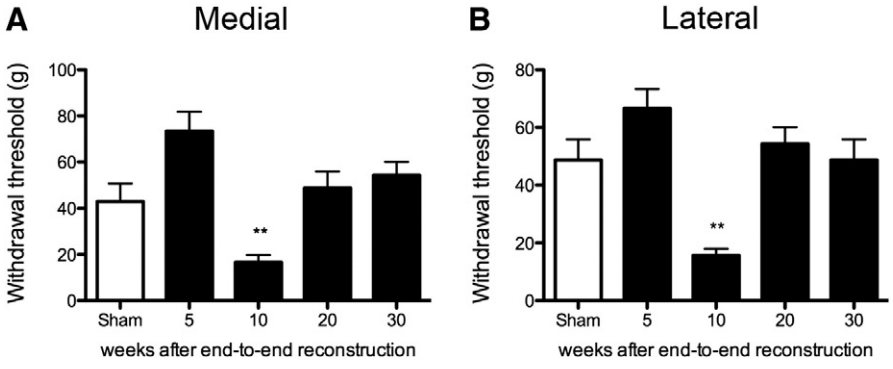

Fig. 1. Contralateral mechanical hypersensitivity. Histograms showing the mechanical withdrawal thresholds in grams (±SEM) in (A) the medial and (B) the lateral region of the contralateral foot sole following unilateral end-to-end reconstruction determined with von Frey monofilaments. A significantly decreased mechanical withdrawal threshold was seen in both the lateral and medial glabrous skin 10 weeks PO in comparison to sham-operated rats. However, the mechanical hypersensitivity diminished 20 and 30 weeks PO. (**: $p < 0.01$, one-way ANOVA).