Sural hypersensitivity after nerve transection depends on anatomical differences in the distal tibial nerve of mice and rats

EM Brakkee [a,*], E. DeVinney [a,b], N. Eijkelkamp [c], JH Coert [a]

[a] Department of Plastic and Reconstructive surgery, University Medical Center Utrecht, Utrecht University, the Netherlands
[b] Axogen, 13631 Progress Boulevard, Alachua, FL 32615, United States
[c] Center for Translational Immunology, University Medical Center Utrecht, Utrecht University, the Netherlands

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ABSTRACT

Introduction: Various mouse and rat models of neuropathic pain after nerve injury exist. Whilst some models involve a proximal nerve lesion or ligation of the *sciatic* trifurcation in mice *and* rats, others consists of a transection or ligation of distal nerves at the *tibial* bifurcation in mice *or* rats. The level of nerve cut directly affects the magnitude of hypersensitivity, and anatomical differences between mice and rats might therefore impact the development of hypersensitivity after distal tibial nerve transection as well.
Methods: The bifurcation of the distal tibial nerve into the medial and lateral plantar nerve (MPN and LPN), and the presence of anatomical differences in sural and tibial nerve distribution between mice and rat was evaluated. Sural mechanical sensitivity after transection of the MPN or whole tibial nerve was assessed using von Frey test until 8 weeks after surgery in 48 rats and 16 mice.
Results: The bifurcation of the tibial nerve into the MPN and LPN is situated proximal to the ankle in both mice and rats. The sural nerve joins the LPN in mice, but not in rats. A proximal communicating branch is present between the LPN and MPN in rats, but not in mice. MPN transection in mice caused hypersensitivity of the hindpaw innervated by the sural nerve, but not in rats. In rats, sural hypersensitivity only developed when both MPN and LPN were cut.
Conclusion: Inter-species variation in nerve anatomy should be taken in consideration when performing surgery to induce plantar hypersensitivity in rodents.

1. Introduction

Neuropathic pain caused by nerve injury is a major clinical problem, that results in loss of function, productivity and quality of life, and can progress into a persisting pain syndrome(Vernadakis et al., 2003). Rodent models are often used to assess putative drugs for the treatment of neuropathic pain after nerve injury(Challa, 2015; Toia et al., 2015). These rodent models with nerve injury typically involve a transection of the tibial nerve to induce mechanical hypersensitivity at the plantar surface of the hind paw innervated by the uninjured sural nerve. Examples of some of these models are the

Spared Nerve Injury (SNI) and Chronic Constriction Injury (CCI) that involve a proximal nerve lesion or ligation at the level of the sciatic trifurcation. Models with a lesion at sciatic level are described in both mice and rats (Fig. 1)(Bennett and Xie, 1988; Bourquin et al., 2006; Cichon et al., 2018; Decosterd and Woolf, 2000; Sant'Anna et al., 2016). In contrast, models that involve a distal transection or ligation at the level of the tibial bifurcation, such as the Distal Nerve Injury (DNI) model, Tibial Neuroma Transposition (TNT) model and Medial Plantar Nerve Ligation (MPNL) model are applied species specific, thus either in mice or in rats (Fig. 1)(Decosterd and Woolf, 2000; Dorsi et al., 2008; Hama and Borsook, 2005). In all above-mentioned models, sural mechanical hypersensitivity (allodynia) is present within one week after surgery. However, in models with a proximal lesion of the sciatic nerve, allodynia tends to be more severe compared to when a more distal lesion of the nerve is performed(Decosterd and Woolf, 2000; Dorsi et al., 2008; Sant'Anna et al., 2016). Thus, the level of nerve cut appears important for the magnitude of hypersensitivity in the uninjured neighboring nerves.

Unfortunately, different nomenclature is being used for the exact location of ligation or transection in models with a distal nerve
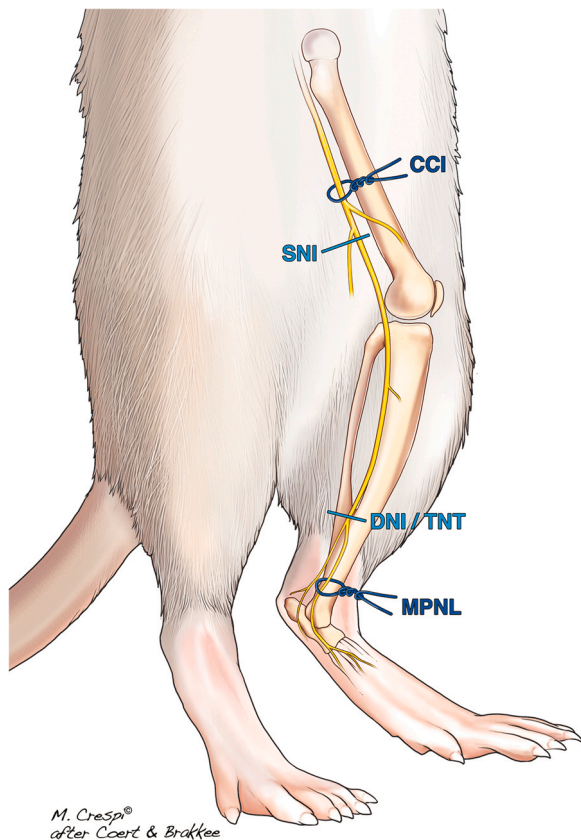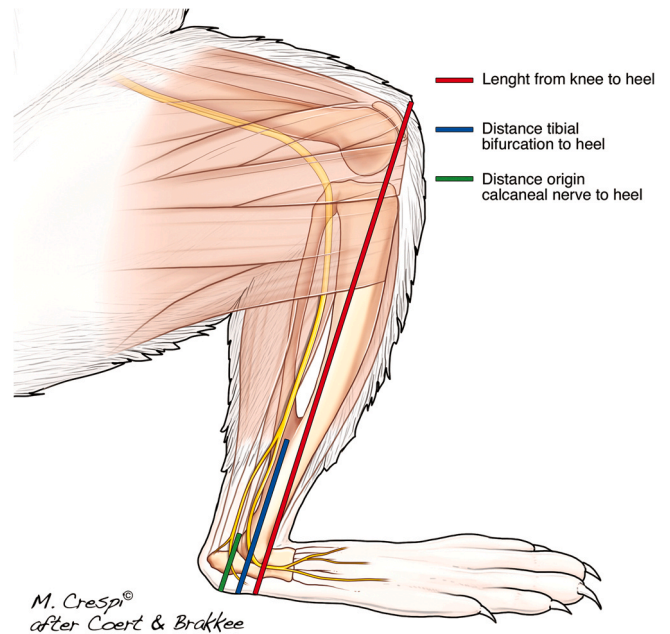
Fig. 1. Example of existing rodent neuropathic pain models. CCI: chronic constriction injury, SNI: spared nerve injury, DNI: distal nerve injury, TNT: tibial neuroma transposition, MPNL: medial plantar nerve ligation. In both mice and rats, the CCI and SNI model are described, which involve a ligation (CCI) or lesion (SNI) at the level of the sciatic nerve. In rats the TNT and DNI model are described, that involve a lesion of the tibial nerve. In mice the MPNL model is described, that involves a ligation of the medial plantar nerve.

Fig. 2. Anatomical measurements in de himdlimb. All measures were done from the heel including skin. The measurements that relate to this figure are shown in Table 1.

lesion, impacting implementation and robustness of the model between labs(Dorsi et al., 2008; Hama and Borsook, 2005; Miyazaki and Yamamoto, 2012). The reason for this variation in nomenclature is unclear, but it seems that in some studies, human anatomy is incorrectly applied on to the rodent situation(Dorsi et al., 2008; Miyazaki and Yamamoto, 2012; Tork et al., 2020). Moreover, if the anatomy of nerves in the distal hindlimb between mice and rats differs as well, this might directly impact the magnitude of hypersensitivity after nerve lesion in both species. Literature is sparse on anatomy of the distal tibial nerve in rodents, and clear anatomical references are essential to guarantee reliability, repeatability and translatability(Moretti et al., 2020). Therefore, we explored the distal tibial nerve and relate the nerve cut to sural hypersensitivity in both mice and rats. In addition, we examined the anatomy of the distal tibial nerve in mice and rats in order to determine model differences between rats and mice and to enhance translatability between species.

2. Methods

2.1. Animals

Mice and rats were housed in groups and kept under a 12:12 h light/dark cycle, with food and water available *ad libitum*. The cages contained environmental enrichments including tissue papers and shelter. This study was approved by the Animal Experimental Commission (DEC) and the Animal Welfare Body (IvD) Utrecht, The Netherlands (AVD1150020198824).

2.2. Anatomical measurements

Surplus mice and rats of other experiments without any surgical history of the hindlimbs were examined under terminal anesthesia. Sixteen hindlimbs of eight C57Bl/6 male mice and sixteen hindlimbs of eight rats (Sprague Dawley (four male, two female) and Lister (two male)) were dissected under a surgical microscope. The hindlimbs were shaved and the total length from knee to heel including skin was measured (Fig. 2). The distance from the plantar side of the heel including skin to bifurcation of the tibial nerve into the medial and lateral plantar nerves (MPN and LPN) was measured (Fig. 2). To differentiate between the LPN and MPN, the nerves were followed by plantar dissection. In a similar fashion, the origin of the calcaneal branch was quantified (Fig. 2). The course of the distal part of the tibial nerve and its plantar branches was photographed and schematically depicted. All anatomical measurements were performed by the same researcher under a surgical microscope in a standardized fashion using a calibrated caliper.

2.3. Surgery

The adult male C57Bl/6 mice (n = 16) and adult male Sprague Dawley rats (n = 48) were randomly divided into five groups: Sham (16 rats and 8 mice), MPN cut (16 rats and 8 mice) and tibial nerve cut (16 rats). Prior to surgery, the animals were anesthetized with 2% isoflurane/O_2. An incision (~1 cm in mice, ~3 cm in rats) between knee and ankle at the medial side of the hindlimb was made. The tibial nerve with the bifurcation into the medial and lateral plantar nerve (MPN and LPN) was identified. In Sham surgery in both mice and rats, the nerves remained untouched and the skin was closed. In MPN cut groups in both mice and rats, the MPN was carefully dissected free, cut and subsequently transposed laterally to prevent regeneration. In tibial nerve cut groups, both the medial and lateral plantar nerves were cut and transposed. Using a 10.0 (mice) or 8.0 (rats) nylon suture, the cut nerves were fixed subcutaneously and the skin was closed. Animals that were included for surgery were checked for any anatomical abnormalities. All surgeries were performed by the same surgeon.

2.4. Sural mechanical hypersensitivity

Animals were acclimatized to the experimental setup for at least 1 week before the start of each experiment, and baseline measurements were at least taken three times. Animals were placed individually into wire mesh-bottom cages and allowed to acclimatize for 15–30 min prior to measurements. Animals were measured for mechanical sensitivity over the sural nerve on the ipsilateral side at baseline and once per week after surgery for 8 weeks. Mechanical sensitivity was assessed by measuring the paw withdrawal threshold in response to a calibrated series of Von Frey hairs ranging from 0.02 g to 4 g (mice) or 0.6–15 g (rats). The filaments were presented perpendicular to the lateral surface of the hind paw innervated by the sural nerve, with sufficient force to cause slight bending or the hair and held for ~3 s. The 50% threshold was defined *via* the up-down method, starting with the 0.4 g (mice) or 4 g (rats) hair (Chaplan et al., 1994; Dixon, 1980). All hypersensitivity measurements were performed blinded to treatment.

2.5. Statistical analysis

Statistical analyses was performed using SPSS version 25.0 and GraphPad Prism version 8.3.0. Graphs were log-transformed and y-axis is changed to the strength of monofilaments. Differences in mechanical hypersensitivity was calculated with a two-way ANOVA and corrected for multiple comparisons using Tukey's test. Differences between groups was measured using an independent sample T-test. A p-value below 0.05 was considered statistically significant.

3. Results

3.1. Anatomical measurements in mice and rats

In mice the length of the lower hind limb from knee to heel was 24 mm (SD 1.0) and 54.6 mm (SD 7.2) in rats. The distance from the heel to the bifurcation of the tibial nerve into the LPN and MPN in mice is 4.7 mm (SD 1.8) and 23.5 mm (SD 8.6) in rats (Table 1 and Fig. 2). In mice, the sural nerve joins the LPN at 1.5 mm (SD 1.7) distal to the bifurcation of the tibial nerve. In rats, the sural nerve does not join tibial nerve branches. A communicating branch between the MPN and LPN proximal to the heel was found in 15/16 rat hindlimbs, and originated from the LPN 9.5 mm (SD2.1) proximal to the heel and joined the MPN at 6.5 mm (SD 1.1) proximal to the heel in all 15 rat hindlimbs (Fig. 3). This communicating branch was not present in all the (16) hindlimbs of mice. The calcaneal branch was identified in 9/16 hindlimbs of mice and in all (16/16) hindlimbs of rats. In mice, some calcaneal branches were likely not identified due to the small diameter of the nerve (Fig. 4). The calcaneal branch originated from the LPN proximal to the heel at 3.1 mm (SD 1.7) in mice and 6.4 mm (SD2.0) in rats. The calcaneal nerve had a significantly smaller diameter than the LPN (Fig. 4).

3.2. *Sural mechanical hypersensitivity in mice*

In mice, transection of the MPN just above the tarsal tunnel induced mechanical hypersensitivity at the hind paw innervated by the sural nerve (Fig. 5A). Mechanical hypersensitivity developed

from 1 week after surgery and was significantly different from sham starting 3 weeks after surgery. The mechanical hypersensitivity persisted at least for up to 8 weeks after surgery (Fig. 5B). As the sural nerve joins the LPN just after the bifurcation, it is not possible to cut the LPN and MPN without damaging the sural nerve, this was therefore not conducted.

3.3. Sural mechanical hypersensitivity in rats

Transection of the MPN just above the tarsal tunnel in rats did not affect mechanical sensitivity at the hindpaw over the sural nerve distribution at all time points measured (Fig. 5C). However, when the whole tibial nerve was cut (both MPN and LPN), hypersensitivity was present from 1 week after surgery and persisted up for the time period measured of 8 weeks after surgery (Fig. 5D).

4. Discussion

4.1. Main results

To our knowledge, the comparative anatomy of the distal tibial nerve between the mice and rats linked to functional outcomes has not been described before. The sural nerve joins the LPN in mice, but not rats. A communicating branch proximal to the heel was present between the LPN and MPN in rats, but not in mice. These differences in anatomy may underlie the development of mechanical hypersensitivity when the MPN is transected. Only in mice, where the sural nerve joins the LPN, mechanical hypersensitivity developed after transection of the MPN. It is known that damage of a nerve leads to hypersensitivity in tissue area innervated by the neighboring nerves. Possibly, because the MPN is only directly neighboring the sural nerve in mice, this may be causal to the species differences in the development of sural hypersensitivity after MPN transection. In rats where the MPN and LPN both neighbors – and not join – the sural nerve, both the MPN and LPN need to be transected in order to establish sural hypersensitivity. We have not examined whether transecting only the LPN results in sural hypersensitivity in rats, because we could not perform a similar surgery for comparison in mice without damaging the sural nerve.

4.2. Translation to human anatomy

Clear description of the anatomy of the distal tibial nerve in rodents is important, because directly applying human anatomy to a rodent model can lead to confusion of nerves to be severed(Banik and Guria, 2021; Dellon and Mackinnon, 1984; Dorsi et al., 2008; Moretti et al., 2020; Warchol et al., 2021). For example, in humans, the bifurcation of the tibial nerve is found near the tarsal tunnel, while we found that in mice and rats, this bifurcation is situated far more proximal in the hindlimb (Dellon and Mackinnon, 1984; Hama and Borsook, 2005). In addition, in humans the calcaneal branch typically origins from the tibial nerve, while we found that in mice and rats it originates from the LPN(Warchol et al., 2021). In humans, there are some reports of communicating branches between the sural and tibial nerve near the feet, however, the complete joining of the sural nerve with the LPN as found in mice, has not been reported in rats or human (Sekiya and Kumaki, 2002). We also found a proximal communicating

Table 1
Mean scores of sixteen dissected lower hindlimbs from mice (n = 8) and rats

Species	Length from knee to heel	Tibial nerve bifurcation	Sural nerve joins LPN	Communicating branch from LPN to MPN	Calcaneal nerve
Mice	24 (1.0)	4.7 (1.8)	3.1 (0.39)	n/a	3.1 (1.7)
Rats	55 (7.2)	24 (8.6)	n/a	9.7 (2.1) > 6.5 (1.1)	6.4 (2.0)

Mean scores measured from the anatomical structure to heel (*e.g.* calcaneal bone with skin) in mm (standard deviation). LPN: lateral plantar nerve, MPN: medial plantar nerve. Distances of the length from knee to heel, tibial nerve bifurcation and calcaneal nerve correspond to the measures shown in Fig. 2.
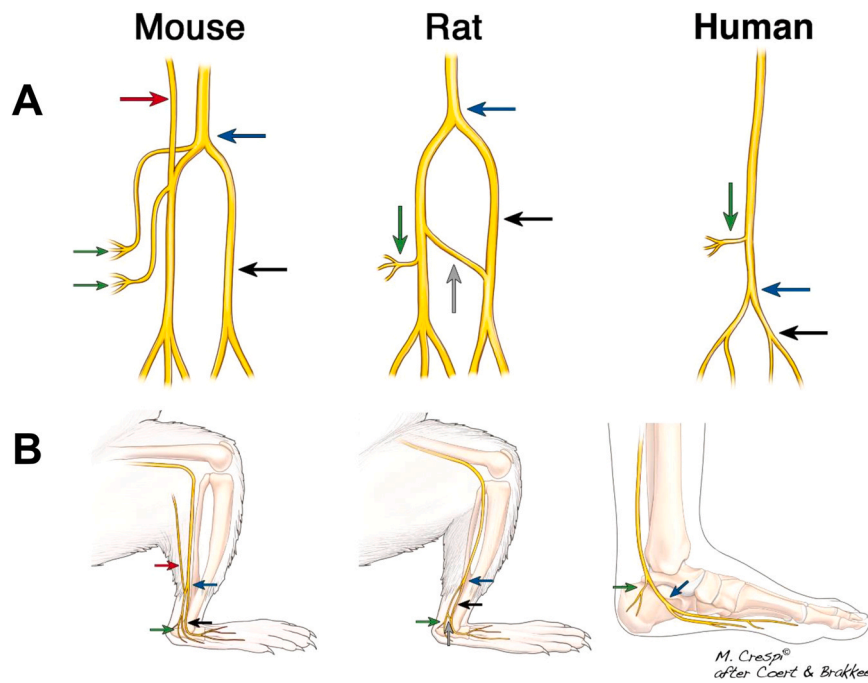
Fig. 3. Comparative anatomy of the tibial nerve with its calcaneal and plantar branches. A: schematic overview of the course of the distal tibial nerve in mouse, rat and human; B: the tibial nerve in relation to bone structures; Arrows: black = medial plantar nerve, blue = bifurcation of the tibial nerve, green = calcaneal branch, gray = communicating branch (rats only), red = sural nerve (mice only). In 9 mice, the calcaneal branch was found and originated proximally to the sural nerve in 6/9 mice and distally in 3/9 mice.

branch between the MPN and LPN in rats. The function of this connection is unknown, but communicating branches like this between the LPN and MPN in humans are usually found more distal in the feet and typically have a purely motoric function, similar to the Martin-Gruber connection between the median and ulnar nerves in humans (Govsa et al., 2005; Kara et al., 2020; Valls-Sole, 1991). Therefore, the proximal communicating branch between the LPN and MPN we found in rats could also have a purely motoric function. Unfortunately, transection of solely the communicating branch and test for functional outcomes in rats is not feasible.

4.3. Animal well-being and autotomy

Analogous to the MPN *ligation* model in mice, we found that *transection* of the MPN leads to sural hypersensitivity in mice as well (Sant'Anna et al., 2016). Our data indicate it is sufficient to only cut the medial plantar nerve to induce hypersensitivity over the sural nerve in mice, thereby preserving innervation of the intrinsic muscles of the paw in contrast to the SNI model, where the intrinsic muscles are paralyzed resulting a contracted and non-functional paw (see supplementary video)(He et al., 2022). Moreover, remaining nociception prevents autotomy – a consequence of anesthesia dolorosa or excessive grooming in absence of sensory feedback(Flecknell, 2002; Koplovitch et al., 2012; Wall et al., 1979). In mice and rats, autotomy occurs after complete peripheral nerve injury in 40–80% of cases, but is rarely described in models with remaining nociception(Koplovitch et al., 2012; Wall et al., 1979; Xu et al., 2021). When autotomy is excessive, humane endpoints potentially cause early termination of experimental studies(Flecknell, 2002). Rodent models with remaining nociception therefore reduce the number of animals needed for experiments. A mice model of
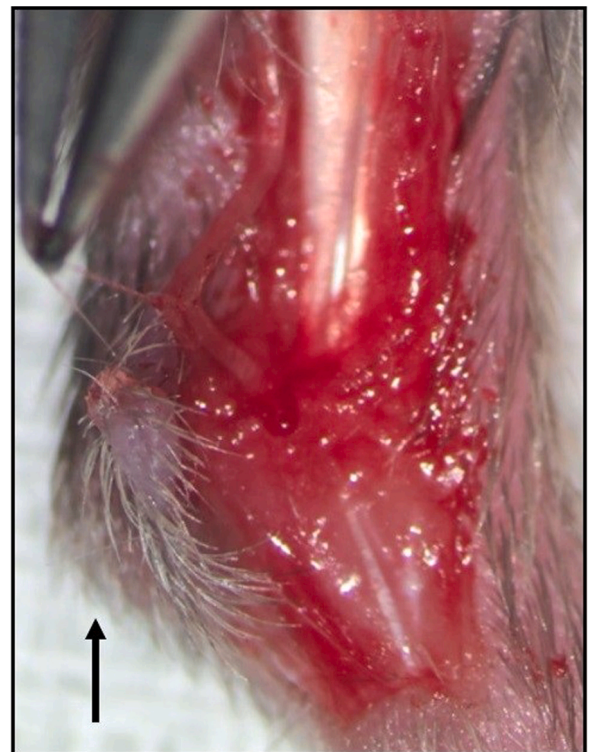
Fig. 4. The calcaneal branch in mice. The bifurcation of the tibial nerve in mice with the calcaneal branch hold with tweezers to the left. The diameter of the calcaneal branch is similar or smaller than that of a mouse hair. Arrow points towards the heel.
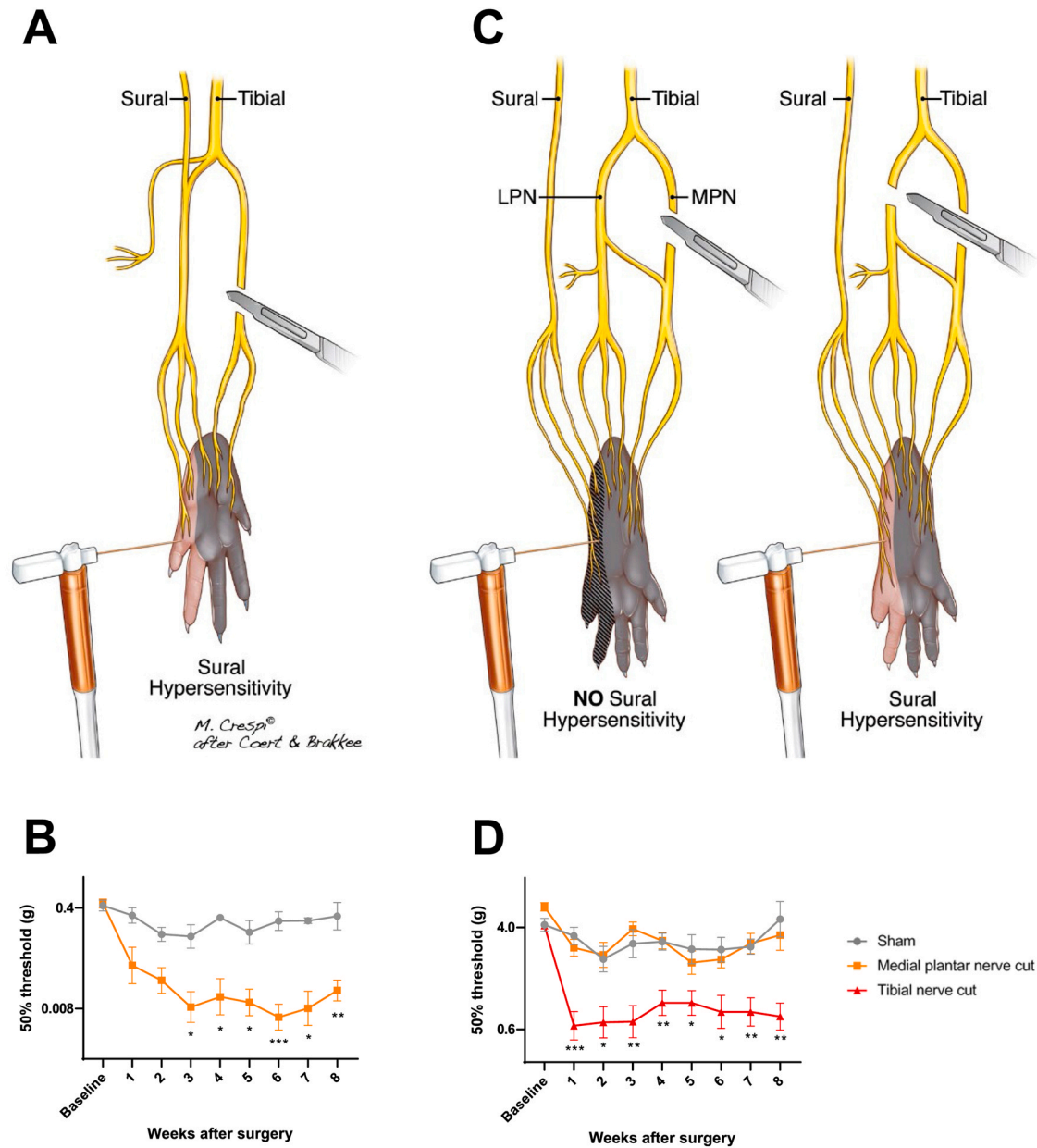
Fig. 5. Mechanical hypersensitivity over the sural nerve after cutting the medial plantar nerve or whole tibial nerve in rats and mice. 5A: schematic representation of the distal tibial nerve in mice with a cut in the MPN, testing with a monofilament is done over the sural nerve distribution. 5B: results of mechanical sensitivity measures in mice, 50% threshold in grams; error bars: standard error of the mean (SEM). Statistics calculated via two-way ANOVA; $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$. 5C: schematic representation of the distal tibial nerve in rats with a cut in the MPN and tibial nerve, testing with a monofilament is done over the sural nerve distribution. 5D: results of mechanical sensitivity measures in rats. 50% threshold in grams; error bars: standard error of the mean (SEM). Statistics calculated via two-way ANOVA; $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$.

sural hypersensitivity caused by MPN transection could potentially be an additional refinement in experimental studies on neuropathic pain after injury.

Supplementary material related to this article can be found on-line at doi:10.1016/j.aanat.2022.152038.

5. Conclusions

Distal tibial branch lesions result in hypersensitivity over the sural nerve, but inter-species variation in distal tibial nerve anatomy should be taken in consideration: Hypersensitivity over the sural nerve can be induced by selectively cutting the MPN in mice, but in rats both plantar branches need to be cut in order to induce sural hypersensitivity.

Ethical statement

This study was approved by the Animal Experimental Commission (DEC) and the Animal Welfare Body (IvD) Utrecht, The Netherlands (AVD1150020198824).

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Conflict of interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors report to have no conflict of interest. Although

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