Intraneural injection of a test dose of local anesthetic in peripheral nerves – does it induce histological changes in nerve tissue?

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Parts of this study were presented at the ESRA 2011, Dresden, Germany

Conflict of interest
TW, B.Braun (advisory board); TS, B.Braun, Teleflex Medical, Vygon (speaker fees); HW, B.Braun, Vygon, Teleflex Medical (speaker fees). All other authors had no potential conflicts of interest

Funding
This work was supported by institutional resources.

Submitted 1 September 2016; accepted 29 September 2016; submission 18 April 2016.

Citation
Wiesmann T, Steinfeldt T, Exner M, Nimphius W, De Andres J, Wulf H, Schwemmer U. Intraneural injection of a test dose of local anesthetic in peripheral nerves – does it induce histological changes in nerve tissue? Acta Anaesthesiologica Scandinavica 2016

doi: 10.1111/aas.12825

Background & Objectives: Most anesthesiologists use the injection of a test dose of local anesthetic in order to evaluate the final needle tip position. Thus, the intraneural injection of a full dose can be avoided. The aim of this study was to analyze whether an intraneural injection of a test dose of bupivacaine could trigger histological changes.
Methods: Intraneural injections under direct vision were performed in 40 brachial plexus nerves in seven anesthetized pigs. Tibial nerves served as positive and negative controls. Two milliliter of bupivacaine 0.5% was injected in three nerves on the left brachial plexus. For control of local anesthetic's toxicity Ringer's solution was applied intraneurally on the right side. After maintaining 48 h of general anesthesia, the nerves were resected. The specimens were processed for histological examination and assessed for inflammation (hematoxylin and eosin stain, CD68-immunohistochemistry) and myelin damage (Kluver–Barrera stain). The degree of nerve injury was rated on a scale from 0 (no injury) to 4 (severe injury).
Results: Statistical analysis showed no significant differences between the bupivacaine group [median (interquartile range) 1 (1–1.5)] and the Ringer's solution group [1 (0.5–2) $P = 0.772$]. Mild myelin alteration was found in 12.5% of all specimens following intraneural injection, irrespective of the applied substance.
Conclusions: "In our experimental study, intraneural injection of 2 ml of bupivacaine or Ringer's solution showed comparable mild inflammation. Nevertheless, inflammation can only be prevented by strictly avoiding nerve perforation followed by intraneural injection, as mechanical nerve perforation is a key factor for evolving inflammation.

Editorial Comment
It is recognized that inadvertant intraneural injection with local anesthetic drugs can lead to nerve injury. In this large animal experimental model, the authors showed that even test dose volumes of local anesthetic, when injected intraneurally, can lead to histopathologically demonstrable nerve inflammation.

Acta Anaesthesiologica Scandinavica
AN INTERNATIONAL JOURNAL OF ANAESTHESIOLOGY AND INTENSIVE CARE, PAIN AND EMERGENCY MEDICINE

Despite widespread implementation of ultrasound-guided peripheral regional anesthesia, accidental intraneural injection as a complication of peripheral nerve anesthesia still occurs in almost 15% of nerve blocks[1], unnoticed even by experts. In order to avoid an unintended full-dose injection of local anesthetic into nerve tissue, it is recommended to inject a small test volume in order to exclude an intraneural needle position. A volume of 0.5–2 ml of saline or local anesthetic is recommended for test dose injection as this is the lower limit of volume that can be noticed by ultrasound experts.[2,3] Additionally, by using a small test dose injection, intravascular injection and injection too distant from the target may be detected.

In addition to the well-studied mechanical trauma caused by needle perforation,[4–6] it is also assumed that an intraneural injection of local anesthetic could induce nerve trauma and neurotoxicity. Previous studies have shown this to be a relevant trigger. Local anesthetics are known to produce relevant apoptosis, necrosis, and inflammation when applied next to peripheral nerves, Schwann, or neuroblastoma cell cultures.[1,7–9] Particularly, signs of inflammation are known as an independent trigger for neurological symptoms,[2–6,10,11] in addition to demyelination effects caused by physical or toxic interferences. However, neuritis following local anesthetic injection has been poorly investigated.

More clinical and experimental data concerning patient safety are required, as the debates concerning intentional 'safe intraneural injections' have evolved.[12–14]

The aim of our experimental study was to analyze whether a low volume of local anesthetic – representing a test dose – could trigger histological changes when injected intraneurally in an established large animal model for peripheral regional anesthesia. We hypothesized that the injection of a 'test dose' of bupivacaine within peripheral nerves would cause pronounced nerve injury when compared to the intraneural injection of Ringer's solution. Primary endpoints of the study were the histological presence and magnitude of post-traumatic regional inflammation and signs of structural injury.

Methods

Animals and anesthesia

Premedication and induction of anesthesia was performed in seven female pigs (Sus scrofa domesticus, 3 to 4 months of age, mean weight 37 kg) after approval by local authorities (Ref. 20-15(1) MR20/13-Nr. 54/2009, RP Giessen, Germany) as previously described.[5,15,16] General anesthesia was maintained using sufentanil (0.5 μg/kg/h, i.v.) and propofol (0.2 mg/kg/min, i.v.) without use of neuromuscular blocking agents. After endotracheal intubation, the animals were ventilated using pressure-controlled ventilation (Siemens Servo 300; Maquet Critical Care, Darmstadt, Germany) with 30% oxygen. Hemodynamics and respiratory function were monitored by ECG, non-invasive blood pressure monitoring (Servomed Monitor; Hellige, Freiburg i.B., Germany), capnometry (DM 8020, Draeger AG, Luebeck, Germany), and pulse oximetry (Biox 3740; Ohmeda, Louisville, CO, USA). Each pig received standardized care in order to maintain sedation for 48 h as described before.[5,16]

Experimental groups

Bupivacaine and Ringer's group

Isobaric bupivacaine 0.5% (Carbostesin 0.5%, isobaric, bupivacainhydrochlorid, AstraZeneca, Hamburg, Germany) or Ringer's solution (Ringer-Lösung B.Braun, B.Braun Medical AG, Melsungen, Germany) were injected into three nerves per side with a diameter of > 2 mm in each brachial plexus (median, radial, ulnar, musculocutaneous, or axillary nerves), respectively. We chose large diameter nerves only to achieve complete intraneural deposit of the injected solutions.

Control groups

Negative controls were applied in order to control for potential confounding effects, such as systemic inflammation following anesthesia or other interventions. Correspondingly, the right tibial nerve of each animal was ligated, serving as a positive control (maximum trauma). The left tibial nerve was not exposed to any intervention, thus serving as a negative control.

Experimental protocol

The surgical exposure of brachial plexus and tibial nerves was performed under aseptic conditions using a standardized approach as described before.[5,15,16] The animals were placed in the supine position with their forelegs abducted. Brachial plexus were exposed surgically to identify each respective nerve and conjoining vessels. Before needle placement, sutures were applied in the surrounding tissue as anatomical references for identification of each injection point at the end of the study. Nerve connective tissue within the plexus sheath was not removed. After execution of scheduled injections, the wounds were closed and dressed aseptically.

The right-hand tibial nerves (serving as positive controls) were surgically exposed and ligated as described, after which the wounds were closed. The left tibial nerves experienced neither surgical exposure nor any other form of intervention, thus serving as negative controls. After 48 h, the wounds were reopened and the nerves were removed for histological examination. The animals were euthanized at the end of the study period by an injection of potassium chloride (4 mmol/kg i.v.)

Injections and test solutions

Intraneural injections were performed with a sterile 24G facet tip syringe, using an immobile needle technique so as to achieve an intraneural injection according to Whitlock et al.[17] The bevel of the needle tip was orientated upwards. For nerve puncturing and needle advancement, an angle of 30° was selected. Injections of 2 ml of fluid were performed within 30 s after a negative aspiration test.

For the purpose of this study, the following solutions were used for injection with bupivacaine (group B) and Ringer's solution (group R):

1. Bupivacaine 0.5% isobaric (Carbostesin 0.5%, Injektionslösung, isobar, Bupivacain-hydrochlorid, Fa. AstraZeneca)
2. Ringer's Solution (Ringer-Lösung B.Braun, B.Braun Medical AG, Melsungen, Germany)

Histology

Nerve specimens were prepared for hematoxylin and eosin staining, Kluver–Barrera staining, and immunohistochemical processing.[5,15] Nerve segments measuring 1–1.5 cm in length were removed and fixed in immersion with formalin. After fixation, the nerve samples were processed for paraffin embedding, and cut into 5 μm slices throughout the whole length. Every fourth slide was stained with hematoxylin and eosin. The initial histological analysis focused on detecting the area of maximal inflammation, followed by a detailed analysis using specific staining for myelin damage and CD68-positive cells. Myelin was stained according to Kluver–Barrera[18] in order to differentiate between vital and non-vital myelin tissue. Immunohistochemical CD68-labelling[19] was applied for the identification of macrophages and monocytes, both key players in evolving neuroinflammation. An established score[4] (Table 1) ranging from 0 (no signs of neural inflammation) to 4 (areas with distinctive signs of inflammation plus myelin damage), facilitated the appraisal of the degree of inflammatory response by a trained observer unaware of the treatment groups.

Sample size calculation and statistics

Assuming a nerve damage score difference of one point between both interventional groups, a standard deviation of 0.5, a power of 0.8, and an alpha-value of 0.05, a minimal sample size of 18 nerves per interventional group was calculated (sample size calculator G*Power, Version 3.1.7, Heinrich Heine University Düsseldorf, Germany). To cope with potential dropouts, 20 nerve injections were scheduled for each interventional group. Negative and positive control groups were allocated, using both tibial nerves of each animal.

Table 1 Nerve injury score.

Score value	Definition
0	No signs of neural injury or inflammation
1	Areas with slight accumulation of inflammatory cells
2	Areas with distinctive signs of inflammation
3	Areas with distinctive signs of inflammation plus hematoma
4	Areas with distinctive signs of inflammation plus myelin damage

Data are presented as median [interquartile range (IQR)]. Due to assumed non-normality of the score value distributions in multiple-group testing, pairwise comparisons of groups were conducted using the Mann–Whitney test (IBM SPSS statistics, release 22.0, IBM, Armonk, USA).

Results

Interventions were performed as scheduled in all seven animals. Twenty nerves in the bupivacaine and Ringer's solution group each were resected. Additionally, seven nerves, as negative and positive controls, respectively, were removed. Two nerves did not receive any intervention as they were potentially damaged during surgical exposure ahead of randomization.

Score values of the bupivacaine group [group B, score 1 (1–1.5)] did not differ significantly from the Ringer's solution group [group R, score 1 (0.5–2), $P = 0.772$]. Negative controls [0 (0–0), $P = 0.002$] and positive controls [4 (4–4), $P = 0.001$] were significantly different compared to the bupivacaine group (Table 2). Relevant myelin damage was found in all nerves in the positive control group, whereas small, localized myelin impairment was found in some of the interventional nerve groups (group R, $n = 3/20$; group B, $n = 2/20$, Table 2). Hematomas were only found in the positive control group (Table 2, Fig. 1). Histological features are displayed in Figs 1–5.

Table 2 Treatment groups.

	Ringer (R)	Bupivacaine (B)	Negative control	Positive control
Nerve specimen (*n*)	20	20	7	7
Score value (median, 25th–75th IQR)	1 (0.5–2)	1 (1–1.5)	0 (0–0)	4 (4–4)
Hematoma (HE) (*n*, specimen)	0	0	0	5
Non-vital myelin (KB) (*n*, specimen)	3	2	0	7

HE, hematoxylin and eosin stain; CD68+, specific staining of CD68-positive leukocytes (macrophages) applying immunohistochemistry; IQR, interquartile range; KB, myelin staining according to the Kluver–Barrera method.

Discussion

Our experimental data show that intraneural injection of a test dose of bupivacaine does not induce more inflammation when compared with a crystalloid solution (Ringer's solution) after a time period of 48 h. Nevertheless, compared with nerves of the negative control group (without intervention) showing no signs of inflammation or myelin damage, both interventional groups had higher trauma scores.

In contrast to cell culture data for Schwann[9] cells and nerve cells[20,21] showing severe apoptosis and necrosis, only mild inflammation and nerve damage were seen in our large animal study.

Selander et al.[22] were the first to investigate the effects of intraneurally injected bupivacaine in anesthetized rabbits (0.05 ml of solution, rabbit weight 2–4 kg). As in our study, partial myelin damage was found in only a few of the investigated nerves. Moreover, this damage was not different when comparing bupivacaine and saline groups. However, Selander et al.[22] showed relevant hematoma after intraneural injection in the majority of the bupivacaine group, which may have resulted in a more pronounced inflammation than in our study, which showed no relevant hematoma in either injection group. Unfortunately, Selander et al. [22] injected a comparably large amount of local anesthetic regarding the size of a rabbit's nerve. Furthermore, they did not investigate evolving local neuroinflammation as a key factor for peripheral neuropathy.[11]

Whitlock et al.[17] showed relevant demyelination after intraneural injection of ropivacaine compared with intraneural saline in a rat model. However, they also did not investigate the inflammatory effects of the applied solutions.

As an important limitation, we did not perform a clinical evaluation in order to correlate histological damage with clinical symptoms as opposed to Hadzic and colleagues.[23] In a large animal experiment, the aforementioned colleagues showed a correlation between injection pressure caused by intraneural needle placement and fascicular injury, resulting in clinical pathologies. However, predictive value of a high injection pressure regarding histological damage and clinical signs of nerve damage in a large animal model are still conflicting,[24] and lacks standardized, validated neurological testing in

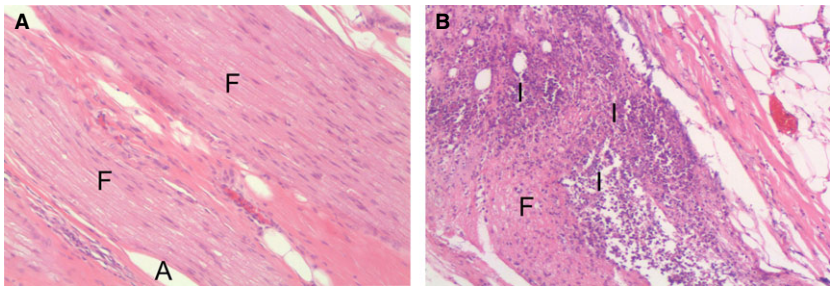

Fig. 1. (A), Histological changes, negative control. Left tibial nerve. Tangential microscopic view (× 200, hematoxylin eosin staining). F, fascicle of nerve; A, artifact. Score value, 0. (B), Histological changes, positive control. Right tibial nerve. Tangential microscopic view (× 200, hematoxylin eosin staining). F, fascicle; I, inflammatory cells. Score value, 4. [Colour figure can be viewed at wileyonlinelibrary.com]

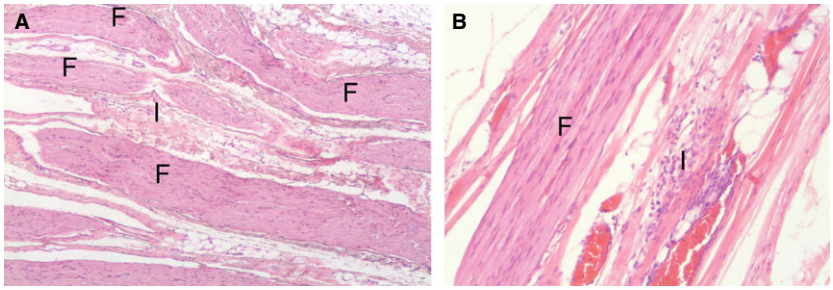

Fig. 2. (A), Histological changes, Group B. Left radial nerve following intraneural injection of bupivacaine 0.5%. Tangential microscopic view (× 100, hematoxylin eosin staining). F, fascicle of nerve; I, inflammatory cells. Score value, 1. (B), Histological changes, Group R. Right musculocutaneous nerve following intraneural injection of Ringer's solution. Tangential microscopic view (× 200, hematoxylin eosin staining). F, fascicle; I, inflammatory cells. Score value, 1. [Colour figure can be viewed at wileyonlinelibrary.com]

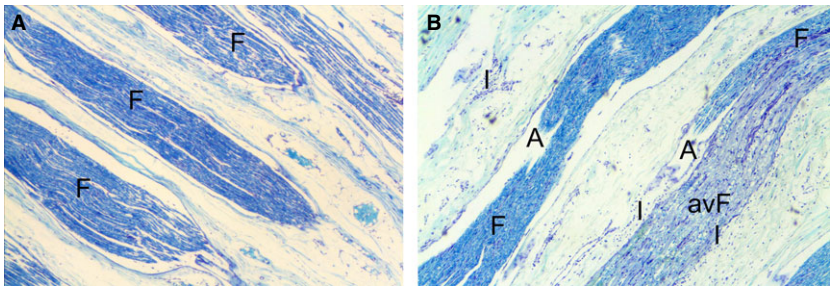

Fig. 3. (A), Histological changes, negative control. Left tibial nerve. Longitudinal microscopic view of the axillary nerve (× 200, staining according to Kluver–Barrera[18]). Myelin appears deep blue according to unaffected myelin. F, fascicle of nerve, A, artifact. Score value, 0. (B), Histological changes, positive control. Right tibial nerve. Longitudinal microscopic view (× 200, staining according to Kluver–Barrera[18]). Myelin appears inconsistent, stretched, swollen and lower stained according to demyelination. F, fascicle of nerve; I, inflammatory cells; A, artifact; avF, avital myelin/fascicle. Score value, 4. [Colour figure can be viewed at wileyonlinelibrary.com]

pigs or dogs.[25] We decided not to evaluate injection pressure as this would not have added further insights into the pharmacologically mediated inflammation caused by bupivacaine. In addition, no electrophysiological examination was performed following the injection for analysis of potentially induced nerve damage. However, the direct electrophysiological testing might interfere with the histological assessment.

Another shortcoming is the limited number of interventional groups and the resulting restriction to one local anesthetic and one crystalloid solution. Other local anesthetics or solutions (e.g., hypotonic dextrose DW-5 solution, saline

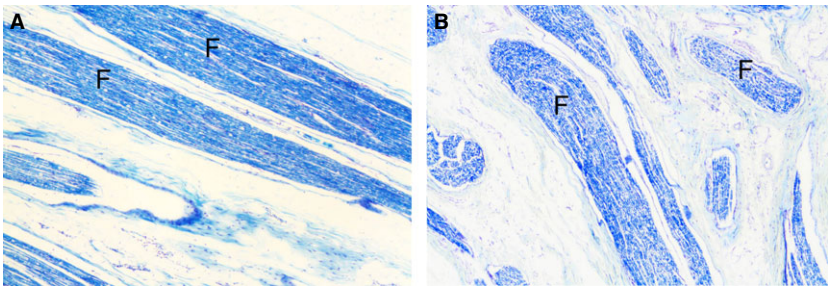

Fig. 4. (A), Histological changes, Group B. Intraneural injection of bupivacaine 0.5%. Longitudinal microscopic view of the radial nerve (× 200, staining according to Kluver–Barrera[18]). Myelin appears deep blue according to an unaffected myelin. F, fascicle of nerve. Score value, 0. (B), Histological changes, Group R. Intraneural injection of Ringer's solution. Longitudinal microscopic view of the axillary nerve (× 200, staining according to Kluver–Barrera). Myelin appears deep blue according to an unaffected myelin. F, fascicle of nerve. Score value, 1. [Colour figure can be viewed at wileyonlinelibrary.com]

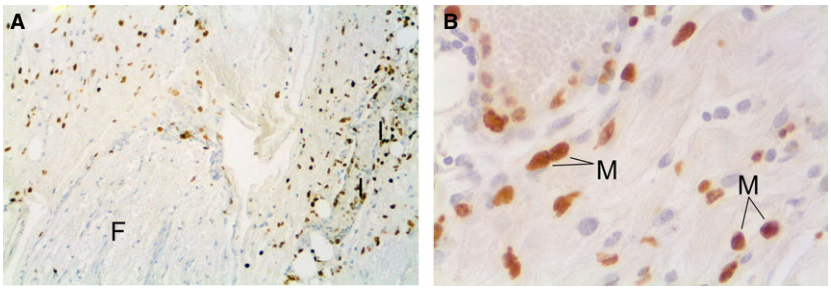

Fig. 5. Trauma-related inflammatory response (immune histochemistry for specific staining of CD68-positive macrophages). I, inflammatory cells; M, macrophages; F, fascicle of nerve. Macrophages are depicted as brown colored immune cells (CD68-positive). Microscopic view of the tibial nerve following ligature. a. Magnification, x 200; b. Magnification, × 400. [Colour figure can be viewed at wileyonlinelibrary.com]

solution) might result in different inflammatory responses. We decided for Ringer's solution instead of saline solution due to potential acidic interferences with nerve tissue. Additionally, we had to limit the number of intervention groups in order to reduce the number of laboratory animals in accordance with local authorities and ethics policies. Therefore, we had no third control group representing nerve perforation only. This topic has been extensively studied by our group as well as other research groups in the past.[4,15,18,19,20] Cumulating published evidence, mechanical needle trauma causing nerve perforation is a key factor in resulting nerve damage.[21] In one of our published reports using comparable methodology, perpendicular nerve perforation using a 24G needle resulted in a nerve damage score of 2 (2/2), which is comparable to the results after using a 24G needle for intraneural injection of both test dose solutions. Therefore, we assume that the type of solution for (low volume) test dose injections may not be relevant, as mechanical nerve perforation without subsequent injection is a key factor for resulting nerve damage.[4] We cannot rule out the possibility that larger volumes might result in higher degree nerve damage as a consequence of aggravating baro- and volutrauma within the peripheral nerve with low compliance.[21]

Additionally, longer follow-up periods than the 48 h in our study may result in different results. However, this would have increased the number of animals used, and was not permitted by local authorities.

Conclusions

Mild nerve inflammation (neuritis) may be triggered by intraneural injection of 2 ml of bupivacaine or Ringer's solution. Mechanical nerve trauma is a key factor for nerve inflammation. Subsequent injection of small volumes (test dose) as a 'second hit' does not seem to boost further inflammation by a pharmacological effect

of the local anesthetic bupivacaine. Considering the fact that no such changes were found in the negative control without mechanical needle trauma, any nerve perforation and subsequent injection should be avoided with regard to the potential of block-related nerve injury.

Acknowledgements

The authors thank Andreas Gockel for his assistance during performance of this study, and Prof. Axel Pagenstecher (Department of Neuropathology, University Hospital Marburg) for his support and facilities during histological examination. Moreover, we thank our colleague Gertrude Duncan for her efforts in language editing.

References

1. Krediet AC, Moayeri N, Bleys RLAW, Groen GJ. Intraneural or extraneural: diagnostic accuracy of ultrasound assessment for localizing low-volume injection. Reg Anesth Pain Med 2014; 39 : 409–13.
2. Eichenberger U, Stöckli S, Marhofer P, Huber G, Willimann P, Kettner SC, Pleiner J, Curatolo M, Kapral S. Minimal local anesthetic volume for peripheral nerve block: a new ultrasound-guided, nerve dimension-based method. Reg Anesth Pain Med 2009; 34: 242–6.
3. Hadzic A. Hadzic's peripheral nerve blocks and anatomy for ultrasound-guided regional anesthesia. NY, USA: McGraw Hill Professional, 2011.
4. Steinfeldt T, Nimphius W, Werner T, Vassiliou T, Kill C, Karakas E, Wulf H, Graf J. Nerve injury by needle nerve perforation in regional anaesthesia: does size matter? Br J Anaesth 2010; 104: 245–53.
5. Steinfeldt T, Graf J, Schneider J, Nimphius W, Weihe E, Borgeat A, Wulf H, Wiesmann T. Histological consequences of needle-nerve contact following nerve stimulation in a pig model. Anesthesiol Res Pract 2011; 2011: 591851.
6. Selander D. Peripheral nerve injury caused by injection needles. Br J Anaesth 1993; 71: 323–5.
7. Hogan QH. Pathophysiology of peripheral nerve injury during regional anesthesia. Reg Anesth Pain Med 2008; 33: 435–41.
8. Neal JM, Gerancher JC, Hebl JR, Ilfeld BM, McCartney CJ, Franco CD, Hogan QH. Upper extremity regional anesthesia: essentials of our current understanding, 2008. Reg Anesth Pain Med 2009; 34: 134–70.
9. Yang S, Abrahams MS, Hurn PD, Grafe MR, Kirsch JR. Local anesthetic Schwann cell toxicity is time and concentration dependent. Reg Anesth Pain Med 2011; 36: 444–51.
10. Eliav E, Benoliel R, Tal M. Inflammation with no axonal damage of the rat saphenous nerve trunk induces ectopic discharge and mechanosensitivity in myelinated axons. Neurosci Lett 2001; 311: 49–52.
11. Eliav E, Herzberg U, Ruda MA, Bennett GJ. Neuropathic pain from an experimental neuritis of the rat sciatic nerve. Pain 1999; 83: 169–82.
12. Bigeleisen PE. Nerve puncture and apparent intraneural injection during ultrasound-guided axillary block does not invariably result in neurologic injury. Anesthesiology 2006; 105: 779–83.
13. Robards C, Hadzic A, Somasundaram L, Iwata T, Gadsden J, Xu D, Sala-Blanch X. Intraneural injection with low-current stimulation during popliteal sciatic nerve block. Anesth Analg 2009; 109: 673–7.
14. Sala-Blanch X, Vandepitte C, Laur JJ, Horan P, Xu D, Reina MA, Karmakar MK, Clark TB, Hadzic A. A practical review of perineural versus intraneural injections: a call for standard nomenclature. Int Anesthesiol Clin 2011; 49: 1–12.
15. Steinfeldt T, Werner T, Nimphius W, Wiesmann T, Kill C, Müller H-H, Wulf H, Graf J. Histological analysis after peripheral nerve puncture with pencil-point or Tuohy needle tip. Anesth Analg 2011; 112: 465–70.
16. Steinfeldt T, Wiesmann T, Nimphius W, Cornelius V, Eismann D, Kratz T, Hadzic A, Wulf H, Werner T. Perineural hematoma may result in nerve inflammation and myelin damage. Reg Anesth Pain Med 2014; 39: 513–9.
17. Whitlock EL, Brenner MJ, Fox IK, Moradzadeh A, Hunter DA, Mackinnon SE. Ropivacaine-induced peripheral nerve injection injury in the rodent model. Anesth Analg 2010; 111: 214–20.
18. Steinfeldt T, Poeschl S, Nimphius W, Graf J, Zoremba M, Mueller H-H, Wulf H, Dette F. Forced needle advancement during needle-nerve contact in a porcine model: histological outcome. Anesth Analg 2011; 113: 417–20.
19. Steinfeldt T, Nimphius W, Wurps M, Eberhart L, Vassiliou T(null), Wulf H, Graf J. Nerve perforation with pencil point or short bevelled needles: histological outcome. Acta Anaesthesiol Scand 2010; 54: 993–9.
20. Selander D, Dhunér KG, Lundborg G. Peripheral nerve injury due to injection needles used for regional anesthesia. An experimental study of the acute effects of needle point trauma. Acta Anaesthesiol Scand 1977; 21: 182–8.

21. Sauter AR, Romundstad L. Animal models can help us prevent nerve injuries in regional anaesthesia for patients. Acta Anaesthesiol Scand 2016; 60: 284–8.
22. Selander D, Sjöstrand J. Longitudinal spread of intraneurally injected local anesthetics. An experimental study of the initial neural distribution following intraneural injections. Acta Anaesthesiol Scand 1978; 22: 622–34.
23. Hadzic A, Dilberovic F, Shah S, Kulenovic A, Kapur E, Zaciragic A, Cosovic E, Vuckovic I, Divanovic KA, Mornjakovic Z, Thys DM, Santos AC. Combination of intraneural injection and high injection pressure leads to fascicular injury and neurologic deficits in dogs. Reg Anesth Pain Med 2004; 29: 417–23.
24. Lupu CM, Kiehl T-R, Chan VWS, El-Beheiry H, Madden M, Brull R. Nerve expansion seen on ultrasound predicts histologic but not functional nerve injury after intraneural injection in pigs. Reg Anesth Pain Med 2010; 35: 132–9.
25. Kapur E, Vuckovic I, Dilberovic F, et al. Neurologic and histologic outcome after intraneural injections of lidocaine in canine sciatic nerves. Acta Anaesthesiol Scand 2007; 51: 101–7.