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Traumatic needle damage to nerves during regional anaesthesia – presentation of a novel mechano-transduction hypothesis

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ABSTRACT

Despite advances in needle positioning techniques, nerve damage still occurs after regional anesthesia. Recognised causes include local anesthetic toxicity, subperineural injection, high subepineural fluid injection pressures, and subepineural haematoma after forceful needle-nerve contact.

We hypothesise that subperineural injection is still possible, but less likely to be the cause of nerve damage because needle penetration of fascicles and mechanical damage is difficult to achieve. High resolution (75 μm) 40-MHz micro-ultrasound images of pig axillae show short-bevelled 22 g, 0.7 mm wide block needles that are three times larger than the average fascicle. Fascicular bundles are extremely difficult to puncture because they spin away on needle contact. Histology from fresh cadavers after supposed intrafascicular injection shows fluid spread within perineurium and intrafascicular perineural septae, but no breach of endoneurium or axons.

We propose that mechano-transduction, the cellular changes that occur in response to force, contributes to nerve damage. Piezo ion channel proteins transduce force into electrical activity by rapid entry of cations into cells. Excessive Ca\(^{2+}\) influx into cells has the potential to inhibit nerve regeneration. Cellular changes include regulation of gene expression. The forces associated with purposeful needle insertion are generally unknown. Our experiments in the soft embalmed Thiel cadaver showed a lognormal range of forces between 0.6 N and 16.8 N on epineural penetration.

We hypothesise that forceful needle injury may cause nerve damage by activation of Piezo receptors and release of intracellular Ca\(^{2+}\).
INTRODUCTION

In this daring discourse we review current perspectives on the nature and mechanisms of nerve injury during regional anesthesia; describe the remarkable ability of peripheral nerves to regenerate; explore recent developments in the field of mechano-transduction; and present a hypothesis of nerve damage, based on laboratory and cadaver-based evidence that challenges established clinical dogma.

Beforehand, we wish to clarify nerve nomenclature as this can be confusing for readers. Therefore, we recommend the following definitions from Reina et al\textsuperscript{1,2} (Table 1). We provide a definition of the axon.

Table 1. Nerve terminology

<table>
<thead>
<tr>
<th>Definition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epimysium</td>
<td>Fascia enclosing muscle, nerves and vessels</td>
</tr>
<tr>
<td>Sub-epimyseal compartment</td>
<td>Tissue between epimysium and either circumneurium or epineurium</td>
</tr>
<tr>
<td>Circumneurium</td>
<td>Fascial layer surrounding two nerves e.g. tibial and common peroneal nerves. Formerly called paraneurium</td>
</tr>
<tr>
<td>Sub-circumneural compartment</td>
<td>Tissue between circumneurium and epineurium</td>
</tr>
<tr>
<td>Epineurium</td>
<td>Tissue layer surrounding nerve. Injection deep to epineurium is termed an intraneural injection.</td>
</tr>
<tr>
<td>Sub-epineural compartment</td>
<td>Tissue between epineurium and perineurium</td>
</tr>
<tr>
<td>Fascicle</td>
<td>Consists of perineurium, endoneurium and axons</td>
</tr>
<tr>
<td>Perineurium</td>
<td>Tissue layer surrounding fascicle</td>
</tr>
<tr>
<td>Subperineural compartment</td>
<td>Tissue deep to perineurium containing endoneurium and axons</td>
</tr>
<tr>
<td>Endoneurium</td>
<td>Tissue surrounding axons</td>
</tr>
<tr>
<td>Axon</td>
<td>A slender projection from the neuronal cell body that enables action potentials to propagate to the nerve terminals at which</td>
</tr>
</tbody>
</table>
Nerve damage

Postoperative neurological symptoms (PONS) may occur in up to 1 in 7 patients after regional anaesthesia. The incidence of PONS reduces exponentially in the year after surgery but persists in approximately 4 in 10,000 patients. The aetiology of nerve damage is complex, and the result of an interplay of several factors. Risk factors may be categorised according to: individual characteristics such as the site of the block and concurrent disease; physical processes such as local anesthetic toxicity, pressure and trauma, and environmental factors such as surrogate markers, equipment and techniques that impact on the accuracy and reliability of injection.

For example: (i) subperineural injection of local anesthetics damaged rat nerves in a concentration, time and zone dependent manner; (ii) traumatic lesions were associated with nerve hematoma in pigs, and (iii) subperineural injection and subepineural injection in dogs generated high pressures in excess of capillary pressures resulting in functional nerve damage.

Given the above, there is a clinical consensus that as long as local anesthetic is injected between and not within fascicles, and that peak pressure remains below a threshold of 15 psi (103 kPa), then injection into nerves should be safe. Testimony to this claim is that no clinical evidence of nerve damage has been recorded while investigating intraneural injection in patients. However, the exact position of the needle tips and the pressures generated at the tip of the needles in these studies were not known.
DISCUSSION

Nerve regeneration

Two principal questions arise. First, what accounts for the exponential reduction in symptomatic neurological side effects in the 12 months after nerve block? Secondly are clinical symptoms representative of nerve damage, or is there underlying nerve damage that is not clinically obvious? The answer may lie in the remarkable capacity of nerves to regenerate after injury. Regeneration is complex and driven by inflammatory and cytotoxic changes\textsuperscript{15,16}. In response to rapid Wallerian degeneration of the distal stump, Schwann cells revert to a progenitor-like state, recruit macrophages for clearance of axonal and myelin debris, and secrete neurotrophic factors that aid nerve growth. The proximal and distal stumps of the damaged nerve are connected by a “bridge” of extracellular matrix, fibroblasts, neutrophils and macrophages. Hypoxic conditions within the bridge stimulate angiogenesis by release of vascular endothelial growth factor (VEGF) from macrophages.

With revascularization, and after clearance of debris, Schwann cells move along the surface of blood vessels and form ordered columns, called the bands of Büngner, that preserve the endoneurial channel and form a regeneration tube for axonal growth. Axonal growth and sprouting are regulated by expression of Regeneration Associated Genes (RAGs) in peripheral neurons. Full recovery, termed maturation, involves remyelination, axon enlargement, and functional re-innervation.

Diagnosis of peripheral nerve injury relies on electrophysiological conduction studies in order to determine the type and severity of nerve damage. Such studies show up to 50% reductions in compound action potentials in both pigs\textsuperscript{17} and patients\textsuperscript{18}. In the latter\textsuperscript{18}, deficits were still present 6 months after regional anaesthesia, but not obvious on clinical examination. However, the results must be placed into context. The researchers used a high and potentially toxic concentration of ropivacaine (10 mg.ml\textsuperscript{-1}) not achieved in standard clinical practice, making these results difficult to interpret. Clinical electrophysiological studies, using appropriate control groups, are needed using much lower concentrations of local anesthetic over a range of nerves, and over a range of patient morbidity in order to evaluate the effect of regional nerve block on nerve function.
Challenging dogma

Challenging clinical dogma surrounding mechanisms of nerve injury has been difficult until now because accurate and reliable identification of subepineural and subperineural injection has been very difficult to simulate in realistic experimental models.

Two advances in imaging technology that identify the precise location of the needle tip and injectate within distinct compartments now provide evidence that challenges the classical theories of nerve damage.

The first imaging advance is the use of heparinised blood marker (HBM) as a histological indicator of injection1. The advantage of HBMs is that erythrocytes are confined to tissue compartments, and thus identify the precise location of injection. In a cadaver study2, ideal circumneural patterns of injection were formed yet HBM was seen in all compartments but not within fascicles. The conclusion of investigators was that intraneural injection may be much more common in clinical practice than otherwise thought, and even contribute to better clinical efficacy19.

The second imaging advance is micro-ultrasound, defined as ultrasound transducer frequencies > 30 MHz. Observation of purposeful needle insertion and nerve injection in anesthetized pigs using real-time, high resolution 40-MHz micro-ultrasound placed in the axilla showed well-defined muscle striation, and sharp, distinct epineurium. The median (IQR) number (n) of fascicles identified using micro-ultrasound was: radial nerve 16 (11 to 19); median nerve 14 (12 to 16); and axillary nerve 8 (6 to 12)20. The tips of standard 22-g block needles, with an external diameter of 0.7 mm, were approximately three times larger than that of the average fascicle and visualized relative to epineurium at resolutions ~ 0.1 mm (Appendix – Video 1)

Attempted intraneural insertion was striking21. One in ten injections failed to penetrate epineurium as nerves rotated in response to forceful contact both in- and out-of-plane. With epineural penetration, fascicular bundles spun on needle tip contact21 and deflected the needle (Appendix – Video 2). On one
occassion, after forceful intraneural needle insertion, perineurial expansion was observed, characterized by the appearance of multiple numbers of round intra-perineural structures (Appendix – Video 3). Histology, similar to Reina et al, showed staining in the perineurium but not any deeper within the endoneurium.

Mechano-transduction

The inflammatory response to intraneural needle insertion and local anesthetic injection is well described. In contrast, the biophysical response to such stressors is less well known. In fact, nerve insertion comprises 3 additive forces - pre-puncture elastic force, cutting force and frictional force, described by equation 1.

Eq. 1. \( f_{\text{needle}}(x) = f_{\text{stiffness}}(x) + f_{\text{friction}}(x) + f_{\text{cutting}}(x) \)

Elastic force displaces and indents the epineurium; cutting force penetrates the epineurium until “cracking” occurs and the needle enters the nerve. Frictional forces increase with continued penetration into the nerve, decrease slowly as tissues relax, and fall rapidly on needle withdrawal\(^{22}\). In addition, it is also important to be aware of the different physical properties of soft tissue that needles penetrate. Tissues may be inhomogeneous (properties depend on location), anisotropic (properties depend on orientation) and viscoelastic.

The conversion of mechanical energy into electrical energy through cellular changes is termed mechano-transduction. It contributes to a range of processes such as proprioception, nociception, development, homeostasis, and disease progression\(^{23}\). Changes in tissue stiffness, shear stress, and pressure can alter cellular processes such as protein synthesis, proliferation, migration, differentiation, and apoptosis. Thus, epineural contact and penetration may represent potential triggers of this process without recourse to subperineural fluid injection. Signal propagation to the nucleus occurs via direct physical interactions of the cell membrane, the cytoskeleton and the nucleus or translocation of
activated mediators from the cytoplasm to the nucleus. These mechanisms couple mechanoreceptors to transcription factors that regulate gene expression.

Mechanoreceptors are potential contributors to the biophysics of nerve injury. Mechanosensitive ion channels, which includes the Piezo channels, are large, complex cell membrane proteins that rapidly transduce mechanical force into electrical signals. Piezo 1 protein, expressed in lungs, bladder and skin, sense bladder distention and regulate blood pressure. Piezo 2 proteins, on the other hand, are expressed in sensory neurons where they are involved in touch, nociception, and proprioception. The importance of piezo channels to life is underscored by the association of mutations in genes encoding Piezo 1 and Piezo 2 channels with severe, incapacitating disease.

Piezo channels, as their name suggests, are activated by force applied to the neuronal membrane, which causes stretch, leading to an inward flow of Na\(^+\) and Ca\(^{2+}\) down their electrochemical gradients. The influx of Ca\(^{2+}\) can directly stimulate Ca\(^{2+}\) sensitive processes, while at the same time cations cause membrane depolarization recruiting voltage-activated Na\(^+\) channels and additional depolarization activating voltage-activated Ca\(^{2+}\) channels.

Excessive or repetitive activation of mechanosensitive ion channels such as the Piezo channels may give rise to excessive Ca\(^{2+}\) entry leading to cellular toxicity that may initiate or contribute to neuronal damage. Recent work demonstrates that the activity of the Drosophila melanogaster Piezo protein inhibits axon regeneration by elevating intracellular Ca\(^{2+}\) and nitric oxide. Neurons have a limited capacity for repair and activation of Piezo channels may impair recovery. By knocking out the drosophila Piezo gene, Song and colleagues demonstrated that the ion channel is responsible for inhibition of axon regeneration following injury. Importantly, they extended their findings into mammals first by investigating axonal regeneration in cultured rat hippocampal neurones and second using laser-mediated nerve trunk ablation of mouse corneal sensory neurones in vivo. Activation of Piezo 1 in the former inhibited axonal regrowth. By contrast, conditional knockout of Piezo 1 in the latter enhanced the rate and extent of axonal regeneration.
Studies in mice have also demonstrated that Piezo 2 channels expressed by Aδ-nociceptors and C-fibres play an important role in noxious mechanosensation\(^27\). Furthermore, both punctate and dynamic allodynia in response to capsaicin-induced inflammation and nerve injury were absent from Piezo 2-deficient mice.

Working out the force required at cellular level in order to activate Piezo channels is challenging. One approach is to apply suction directly to the cell membrane using a patch-clamp recording electrode in the cell-attached patch recording configuration\(^25\). Using this approach, it has been established that a pressure of approximately 10 mm Hg is sufficient to reach threshold and activate Piezo-mediated currents\(^28\). Recent laboratory evidence demonstrates that Piezo channels also enhance mechanical activation and modify the biophysical characteristics of tandem two pore K\(^+\) channels through local depletion of membrane cholesterol\(^29\). Furthermore, Piezo 1 upregulates TREK-1/2 channels in mouse fibroblasts causing delayed wound healing.

In contrast, at a macroscopic level, needle insertion on soft embalmed Thiel cadavers demonstrated a wide range of log-normal distributed forces between 0.3 N and 9.3 N on epineural contact and 0.6 N and 16.8 N on epineural penetration that, importantly, did not correlate with paired fluid injection pressure measurements\(^30\). Lack of correlation occurs because fluid injection pressure and force represent different components of needle-nerve interaction separated by time. Needles inserted percutaneously generate an axial force that displaces tissues, whereas pressure is measured by injection of local anaesthetic at a constant rate, but only when tissues are static.

Given our argument above, we hypothesize that Piezo channels are activated during needle nerve contact and nerve penetration as well as subepineural and subperineural injection. Thus, forceful intraneural needle insertion may be risky in the absence of an intrafascicular puncture. We further hypothesize that force applied to nerves above an unknown threshold activates inward flow of cations triggering biochemical pathways deleterious to nerve function and integrity. Mechano-transduction
may also lead to aberrant Schwann cell function, imbalance of the immune and inflammatory response to nerve degeneration and regeneration after injury potentially contributing to permanent damage, impaired nerve function and chronic pain. Understanding these processes may have implications for needle-nerve contact in clinical practice.

In conclusion, we hypothesise that subperineural injection is less likely than currently thought to be a cause of nerve damage because needle penetration of fascicles is difficult to achieve. Fascicular bundles rotate in response to contact and perineural injection, and expansion of septae into fascicles mimics endoneural and fascicular injection. We suggest that forceful needle injury causes nerve damage by activation of Piezo receptors and release of intracellular Ca²⁺. We intend to measure the forces associated with needle insertion in patients.

REFERENCES

2 Reina MA, Sala-Blanch X, Monzo E, Nin OC, Bigeleisen PE, Boezaart AP. Extrafascicular and Intraperineural, but No Endoneural, Spread after Deliberate Intraneural Injections in a Cadaveric Study. Anesthesiology 2019; 130: 1007-16
12 O'Flaherty D, McCartney CJL, Ng SC. Nerve injury after peripheral nerve blockade-current understanding and guidelines. BJA Educ 2018; 18: 384-90
15 Huebner EA, Strittmatter SM. Axon regeneration in the peripheral and central nervous systems. Results Probl Cell Differ 2009; 48: 339-51
19 Boezaart A, Sala-Blanch X, Monzo E, Reina MA. Our best anesthetic blocks are probably related to unintentional and unnoticed intraneural injection. Reg Anesth Pain Med 2019
22 Okamura AM, Simone C, O'Leary MD. Force modeling for needle insertion into soft tissue. IEEE Trans Biomed Eng 2004; 51: 1707-16
27 Murthy SE, Loud MC, Daou I, et al. The mechanosensitive ion channel Piezo2 mediates sensitivity to mechanical pain in mice. Sci Transl Med 2018; 10
Appendix

Video 1

Right median nerve lying below pectoral muscle. Depth of image 9mm. 21g short bevelled B.Braun Simuplex needle seen approaching and entering nerve. Once in nerve a 0.5ml hydrolocation test dose is injected and swelling apparent in lower pole of nerve. Once the needle is removed, the nerve returns back to similar size and configuration.

Video 2

From left to right: vein, artery beneath median nerve, axillary nerve and radial nerve. Size 21g B.Braun Simuplex needle inserted and targeting axillary nerve. Note mobility of axillary nerve between 33s and 48s and split within axillary nerve at 54s

Video 3

Intraneural injection into region around right lower axillary and median nerves. Initial localised swelling occurred in the left axillary nerve at 8s followed by swelling within perineurium of median nerve at 9s.

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AS writing—review and editing of the manuscript
TGH conception and writing—review and editing of the manuscript

All authors critically revised the manuscript. All authors approved the final version of the manuscript.

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