Effects of experimental focal compression on excitability of human median motor axons

Tatsunori Ikemoto[a], Toshikazu Tani[a], Shinichirou Taniguchi[a], Masahiko Ikeuchi[a], Jun Kimura[b]

[a]*Department of Orthopaedic Surgery, Kochi Medical School, Kohasu Oko-cho, Nankoku City, Kochi 783-8505, Japan*

[b]*Department of Neurology, University of Iowa, Hospitals and Clinics, Iowa City IA 52242-1053, USA*

Key words: Nerve compression, Nerve excitability, Threshold tracking, Ischemia, Membrane depolarization

Abstract

Objective: To characterize the effect of focal compression by threshold tracking and other excitability measures of human median motor axons.

Methods: We conducted a sequence of excitability studies using a software written in BASIC (QTRAC version 4.0, ©Institute of Neurology, London, UK, with multiple excitability protocol TRONDXM 2) in 24 healthy subjects, stimulating the median nerve at the wrist and recording compound muscle action potentials from the abductor pollicis brevis. Constant, localized compression was applied at the wrist by mechanically lowering a probe attached to a disk electrode, which also served as the stimulating cathode.

Results: Compared with the pre-compression values, measurements during compression showed a shift of threshold electrotonus waveforms toward the baseline (fanning-in), steeper current-threshold relationships, increased strength-duration time constants, prolonged relative refractory periods and reduced levels of superexcitability, but no alteration in late subexcitability. These excitability changes indicating depolarization reversed to hyperpolarization immediately after release of compression. The nerve compression altered none of the excitability measures when recorded 2 cm distally from the pressure probe.

Conclusions: Mild nerve compression produces a very localized axonal depolarization at the compression site followed by hyperpolarization upon release of compression, as expected from focal ischemia.

Significance: The current results imply that the sharply-localized conduction abnormalities demonstrated electrophysiologically in peripheral nerve entrapment syndromes and compression myelopathies may, in part, result from

compression-induced focal nerve ischemia.

1. Introduction

Among various nerve excitability studies, threshold electrotonus waveforms provide a particularly sensitive measure of membrane potential, elucidating sub-threshold electrical properties of the axon (Bostock *et al.*, 1998). Studying the effect of brief periods of pressure cuff inflation with this technique, Bostock et al. (1991) found a greater ischemic change underneath the cuff than below it. This result suggested the etiological importance of exclusion of blood from the nerve rather than mechanical deformation for compression symptoms.

In the current study, we wished to delineate if the same mechanisms apply to a focal nerve compression induced by a specially constructed pressure probe, which serves as a model of entrapment neuropathy. Using this device, we have conducted multiple nerve excitability studies including measurements of threshold electrotonus of the median motor axons, comparing compression-induced changes to previously reported effect of nerve ischemia (Bostock et al., 1998; Kiernan and Bostock, 2000; Kaji, 2003).

2. Materials and methods

2.1. Subjects

We studied the left median nerves in 24 healthy subjects (14 males), aged 22-55 years (mean: 28 years) after obtaining a written informed consent. Of these, 17 subjects underwent excitability studies at the compression site, 10, during compression and 7, after release of compression, whereas the remaining 7 subjects had the effect of compression tested 2 cm distal to the pressure probe.

The study was approved by the Kochi Medical School Ethics Committee.

2.2. Nerve compression

The subject lay supine on a table with the left forearm supinated on the base plate of a specially-designed external compression device as described earlier (Tani *et al*., 2001) (Fig 1). Localized compression was applied to the median nerve by lowering a probe attached to a disk electrode placed over the nerve at the wrist. The same disk electrode was used as the stimulating cathode to test the membrane properties of the compressed nerve segment with the threshold tracking techniques (Bostock et al., 1998; Kiernan et al., 2000). A constant load of 12 N was applied to the nerve for 20 minutes, lowering the pressure probe vertically.

2.3. Stimulation

The disposable discs were used for monopolar stimulation measured 12 mm in diameter (Vitrode J-150, Nihonkohden, Tokyo, Japan), although the effective electrode surface extends further when attached to the skin with electrolyte. The cathode was placed over the nerve 4 cm proximal to the distal crease of the wrist, and anode 10 cm further proximally on the forearm.

2.4. Recording

A set of 11 mm surface electrodes (NE-132B, Nihonkohden) was placed optimally over the thenar eminence to register compound muscle action potentials (CMAPs) primarily from the abductor pollicis brevis. A ground strap, 2 cm in width, was wrapped around the wrist or the hand. The forearm skin temperature was maintained 35±0.5 °C

throughout the experiment with a thermostatically-controlled heat lamp coupled to a thermistor probe.

Stimulation and recording were controlled by a software, written in BASIC (QTRAC version 4.0, ©Institute of Neurology, London, UK, with multiple excitability protocol TRONDXM 2), designed to test a number of different nerve excitability measures (Kiernan *et al*., 2000). Excitability studies included: (1) the current-threshold relationship tested with 1 ms pulses at the end of 200-ms polarizing currents, which were altered from +50% (depolarizing) to -100% (hyperpolarizing) of the control threshold in 10% steps; (2) the strength-duration time constants estimated from thresholds at two different stimulus durations of 0.2 ms and 1 ms based on Weiss's law (Mogyoros, *et al*., 1996); (3) threshold electrotonus determining the time course of membrane excitability change induced by a 100-ms sub-threshold depolarizing or hyperpolarizing current pulse (±40% of the control threshold current) based on the intensity of the test shock of 1 ms duration necessary to evoke 40% of the maximal response; (4) the recovery cycle of excitability following a supramaximal conditioning stimulus of 1 ms duration investigating the relative refractory period, superexcitability and late subexcitability. The relative refractory period, during which a test stimulus must be abnormally large to evoke a second response, was defined as the interstimulus interval at which threshold recovered to its control value. The superexcitability was measured as the greatest percentage reduction in threshold and late subexcitability, as the greatest percentage increase in threshold following a period of superexcitability.

A sequence of excitability measurements was recorded twice; baseline and during compression, starting 10 minutes after the pressure was applied, or immediately after release of 20 minute compression. We tested the recovery cycle in the final part of the

recording session lasting 10-15 minutes. This implies that observed changes of excitability must sustain at least up to 10 minutes after the pressure is removed. Values are given as mean±SD. We used Wilcoxon's signed rank test to compare and contrast values for the excitability measures with studies in the pre-compression period and during or after compression, considering two tailed tests significant when $p<0.05$.

3. Results

Figure 2 and Table 1 show compression-induced axonal excitability changes at the compression site (filled circles in the figure) and the baseline data obtained prior to compression (open circles in the figure) in 10 subjects. The current-threshold relationship (Fig 2-A) became significantly steeper during compression compared with the pre-compression curve ($p=0.007$ for 50% polarizing currents), indicating compression-induced depolarization with increased outward rectification for depolarizing conditioning current. Strength-duration time constants (Fig 2-B) significantly increased ($p=0.007$) for every fraction of the CMAP during compression compared with the pre-compression values. Figure 2-C shows the threshold changes induced by a 100-ms sub-threshold depolarizing or hyperpolarizing current pulse. The recordings, which started 10 minutes after compression, shifted toward the baseline (fanning-in) compared with the pre-compression response curves, indicating membrane depolarization with compression.

For statistical analysis, we compared the threshold changes from 90 to 100 ms after the start of a 100-ms sub-threshold depolarizing current pulse (TEd [90-100 ms]) or a hyperpolarizing current pulse (TEh [90-100 ms]) with the data obtained during

compression and prior to compression. The values were significantly smaller (p=0.007) during compression for both TEd (35.5±6.2% vs 43.6±4.8%) and TEh (-69.5±19.9% vs -104.8±18.7%) than those prior to compression. Figure 2-D shows the recovery cycle of excitability. During compression, the relative refractory period increased (p=0.007) and superexcitability decreased (p=0.007) compared with those before compression. In contrast, late subexcitability remained unaltered (p=0.33) during compression.

Figure 3 and Table 1 show axonal excitability changes at the site where compression was just released (filled circles in the figure) and the baseline data obtained prior to compression (open circles in the figure) in 7 subjects. Release of compression reversed excitability properties of the axons, which now showed changes consistent with membrane hyperpolarization compared to the pre-compression curve as follows. The current-threshold relationship (Fig 3-A) became less steep (p=0.028) with decreased outward rectification for depolarizing conditioning current. Strength-duration time constants (Fig 3-B) showed a significant decrease (p=0.028) for every fraction of the CMAP. The response curves of threshold electrotonus (Fig 3-C) shifted away from the baseline (fanning-out), showing greater values (p=0.028) for both TEd (55.3±5.5% vs 44.9±1.4%) and TEh (-146.9±9.6% vs -120.6±8.9%). The recovery cycle of excitability (Fig 3-D) also reversed, showing a decrease in relative refractory period (p=0.028), an increase in superexcitability (p=0.028) and a decrease in late subexcitability (p=0.028), all consistent with membrane hyperpolarization.

In contrast, the nerve compression altered none of the excitability measures when recorded 2 cm distally from the pressure probe (Figs 4 A-D and Table 1).

4. Discussion

We have previously shown that localized compression of the median nerve initially gives rise to a substantial diminution in size of orthodromic compound sensory nerve action potentials without notable change in latency (Tani *et al*., 2001). This finding, indicating preferential vulnerability of slower myelinated fibers, suggested compression-induced ischemia, which, in animal studies, affects the smaller myelinated fibers before the larger fibers (Parry *et al*., 1985; Fern and Harrison, 1994a,b). Accordingly, we postulated that this type of nerve insult may result from focal perineural ischemia directly under the localized compression,

In the current study, we have shown that a focal compression induces 'fanning-in' in threshold electrotonus (Fig 2-C), longer strength-duration time constants (Fig 2-B), an increased relative refractory period and a reduced superexcitability (Fig 2-D), all characteristics of a depolarized axonal membrane. The results are consistent with earlier work (Kiernan et al., 1999) which demonstrated that maintained wrist extension increased refractoriness and decreased superexcitability of the median nerve at the wrist, suggesting axonal depolarization.

Analysis of the late subexcitable phase of the recovery cycle can help distinguish three different mechanisms of depolarization (Kiernan *et al*., 2000, 2002): (1) DC depolarizing current, which enhances late subexcitability by activating slow K^+ channels and increasing K^+ efflux (Dubois, 1981; Kiernan *et al*., 2000); (2) hyperkalemia, which reduces late subexcitability by increasing K^+ influx according to the concentration gradient (Kiernan *et al*., 2002); and (3) ischemia, which tends to cause the late subexcitability to remain unchanged (Kiernan *et al*., 2000) possibly by two opposing factors: inactivation of the electrogenic sodium pump and extracellular K^+ accumulation (Bostock *et al*., 1998), although additional factors may also play a role.

Regardless of the underlying mechanisms, a normal late subexcitability implies an ischemic basis for our finding of compression-induced depolarization.

A reversal of membrane properties from depolarization to hyperpolarization after release of compression also has a striking resemblance to tourniquet-induced ischemia (Bostock et al., 1998; Kiernan and Bostock, 2000; Kaji, 2003) with the exception of reduced late subexcitability seen in our study. Bostock *et al*. (1991), investigating the time course of post-ischemic changes in human motor axons, demonstrated two consecutive stages of membrane excitability following ischemia, earlier, hyperpolarized state and later less hyperpolarized state. A rapidly increasing sodium pump activity seen in the first stage probably plays a greater role in our experiment conducted immediately after the removal of compression. If so, axonal hyperpolarization would decrease K^+ efflux, reducing late subexcitability, as observed in our study. Additionally, superexcitability may have extended into the conditioning-test intervals normally associated with maximal late subexcitability, extrapolating from the previous observation of the reciprocal nature of the ischemic changes in supernormality and refractoriness (Grosskreutz et al., 2000). In contrast, reduced hyperpolarization seen in the second stage should characterize the measures obtained 5 minutes after releasing a pressure cuff, when a decreased K^+ efflux would abate, giving rise to a physiological late subexcitability as reported by Kiernan and Bostock (2000).

In our study, nerve compression had its effect on the axon restricted to the site of insult as evidenced by unaltered excitability properties measured 2 cm distally. Animal experiments on nerve compression also revealed a reduction in nerve blood flow in association with increased endoneurial fluid pressure at the site of compression (Rydevic *et al*., 1981; Myers *et al*., 1982; Lundborg *et al*., 1983). Ischemia has effects

on axonal excitability that cannot be entirely explained by changes in membrane potential (Grosskreutz et al., 2000). Metabolic products of ischemia may interfere with the recovery of Na^+ channels from inactivation, inducing a rapidly reversible conduction block seen in our previous experiments (Tani et al., 2001; Tsuboya et al., 2007). All these findings considered together imply that the sharply-localized conduction abnormalities demonstrated electrophysiologically in peripheral nerve entrapment syndromes (Kimura, 1979; 2001) and compression myelopathies (Tani *et al*, 1998, 1999, 2002, 2006) may, in part, result from focal nerve ischemia (Kiernan et al., 1999).

We conclude that compressing the nerve by a small probe causes focal ischemia, which in turn induces a very localized, reversible axonal depolarization directly under the compression followed by hyperpolarization upon release of compression. This would account for a very rapid return of function immediately after surgical decompression in some cases of entrapment neuropathy and spondylotic myelopathy (Ishida *et al.*, 2003).

References

Bostock H, Baker M, Grafe P, Reid G.. Changes in excitability and accommodation of human motor axons following brief periods of ischaemia. J Physiol 1991;441,513-535.

Bostock H, Baker M, Reid G.. Changes in excitability of human motor axons underlying post-ischaemic fasciculations: evidence for two stable states. J Physiol 1991;441,537-557.

Bostock H, Cikurel K, Burke D. Threshold tracking techniques in the study of human peripheral nerve. Muscle Nerve 1998;21:137-158.

Dubois JM. Evidence for the existence of three types of potassium channels in frog Ranvier node membrane. J Physiol (Lond) 1981;318:297-316.

Fern R and Harrison PJ. The relationship between ischaemic conduction failure and conduction velocity in cat myelinated axons. Exp Physiol 1994a;79:571-581.

Fern R and Harrison PJ. The contribution of ischaemia and deformation to the conduction block generated by compression of the cat sciatic nerve. Exp Physiol 1994b;79:583-592.

Grosskreutz J, Lin CSY, Mogyoros I, Burke D. Ischaemic changes in refractoriness of human cutaneous afferents under threshold-clamp conditions. J Physiol 2000;523.3:807-815.

Ishida K, Tani T, Ushida T, Zinchuk VS, Yamamoto H: Recovery of spinal cord conduction after surgical decompression for cervical spondylotic myelopathy: serial somatosensory evoked potential studies. Am J Phys Med Rehabil 82,130-136, 2003.

Kaji R. Physiology of conduction block in multifocal motor neuropathy and other demyelinating neuropathies. Muscle Nerve 2003;27:285-296.

Kiernan MC, Mogyoros I, Burke D. Conduction block in carpal tunnel syndrome. Brain 1999;122:933-941.

Kiernan MC, Bostock H. Effects of membrane polarization and ischaemia on the excitability properties of human motor axons. Brain 2000;123:2542-2551.

Kiernan MC, Burke D, Andersen KV, Bostock H. Multiple measures of axonal excitability: a new approach in clinical testing. Muscle Nerve 2000;23:399-409.

Kiernan MC, Walters RJI, Andersen KV, Taube D, Murray NMF, Bostock H. Nerve excitability changes in chronic renal failure indicate membrane depolarization due to hyperklaemia. Brain 2002;125:1366-1378.

Kimura J. The carpal tunnel syndrome : Localization of conduction abnormalities within the distal segment of the median nerve. *Brain* 1979;102:619-35.

Kimura J. Electrodiagnosis in diseases of nerve and muscle: principles and practice. 3rd ed. New York: Oxford University Press, 2001:178-214.

Lundborg G, Myers R, Powell H. Nerve compression injury and increased endoneurial fluid pressure: a “miniature compartment syndrome”. J Neurol Neurosurg Psychiatry 1983;46:1119-1124.

Mogyoros I, Kiernan MC, Burke D. Strength duration properties of human peripheral nerve. Brain 1996;119:439-447.

Myers RR, Mizisin AP, Powell HC, Lampert PW. Reduced nerve blood flow in hexachlorophene neuropathy: relation to elevated endoneurial fluid pressure. J Neuropathol Exp Neurol 1982;41:391-399.

Parry GJ, Cornblath DR, Brown MJ. Transient conduction block following acute peripheral nerve ischemia. Muscle Nerve 1985;8:409-412.

Rydevik B, Lundborg G, Bagge U. Effects of graded compression on intraneural blood flow. J Hand Surg 1981;6:3-12.

Tani T, Ushida T, Yamamoto H, Kimura J: Waveform analysis of spinal somatosensory evoked potential: paradoxically enhanced negative peak immediately caudal to the site of conduction block. Electroencephalogr Clin Neurophysiol 108 : 325-330, 1998.

Tani T, Yamamoto H, Kimura J: Cervical spondylotic myelopathy in elderly people: a high incidence of conduction block at C3-4 or C4-5. J Neurol neurosurg Psychiatry 66: 456-464, 1999.

Tani T, Ushida T, Kimura J. Sequential changes of orthodromic sensory nerve action potentials induced by experimental compression of the median nerve at the wrist. Clin Neurophysiol 2001;112:136-144.

Tani T, Ushida T, Taniguchi S, Kimura J: Age related shift in the primary sites of involvement in cervical spondylotic myelopathy from lower to upper levels. J Neurol Neurosurg Psychiatry 73: 316-318, 2002.

Tani T, Ushida T, Taniguchi S, Ishida K, Tsuboya H, Ikemoto T: Partial conduction block in cervical compression myelopathies: waveform changes of ascending spinal evoked potentials. Suppl Clin Neurophysiol 59, 265-274, 2006.

Tsuboya H, Tani T, Ishida K, Ushida T, Taniguchi S, Kimura J. Quantitative sensory testing of cold and vibration perception during compression of median nerve at the wrist. Muscle Nerve 35, 458-464, 2007.

Fig 1. Semischematic drawing of the general experimental design. The CMAPs were recorded with surface electrodes placed over the belly and tendon of the abductor pollicis brevis after stimulation of the median nerve at the wrist. Localized compression was applied to the median nerve by lowering the compressing probe to press the stimulating disk electrode (cathode) vertically with a constant load of 12 N. The remote stimulating electrode (anode) was placed over muscle 10 cm proximal to the cathode.

Fig 2. Compression-induced axonal excitability changes at the compression site (filled circles) and the baseline data obtained prior to compression (open circles) in 10 subjects. (A) Current-threshold relationship. (B) Strength-duration time constants for 9 fractions of the CMAPs. (C) Threshold electrotonus. (D) Recovery cycle of excitability.

Fig 3. Axonal excitability changes at the site where compression was just released (filled circles) and the baseline data obtained prior to compression (open circles) in 7 subjects. (A) Current-threshold relationship. (B) Strength-duration time constants for 9 fractions of the CMAPs. (C) Threshold electrotonus. (D) Recovery cycle of excitability.

Fig 4. Axonal excitability changes of the nerve segment 2 cm distal to compression (filled circles) and baseline data obtained prior to compression (open circles) in 7 subjects. (A) Current-threshold relationship. (B) Strength-duration time constants for 9 fractions of the CMAPs. (C) Threshold electrotonus. (D) Recovery cycle of excitability.

Figure. 1

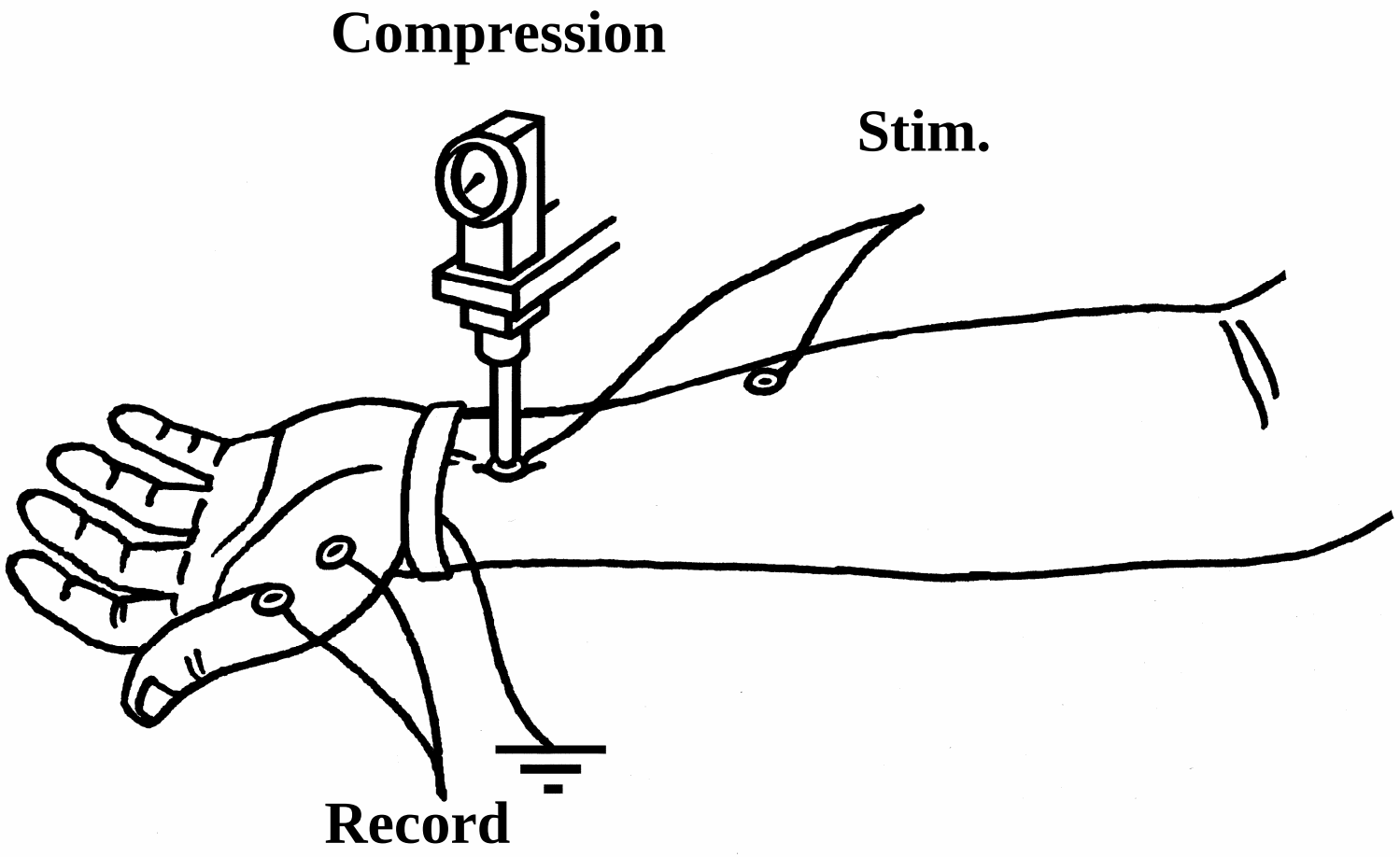

Figure. 2

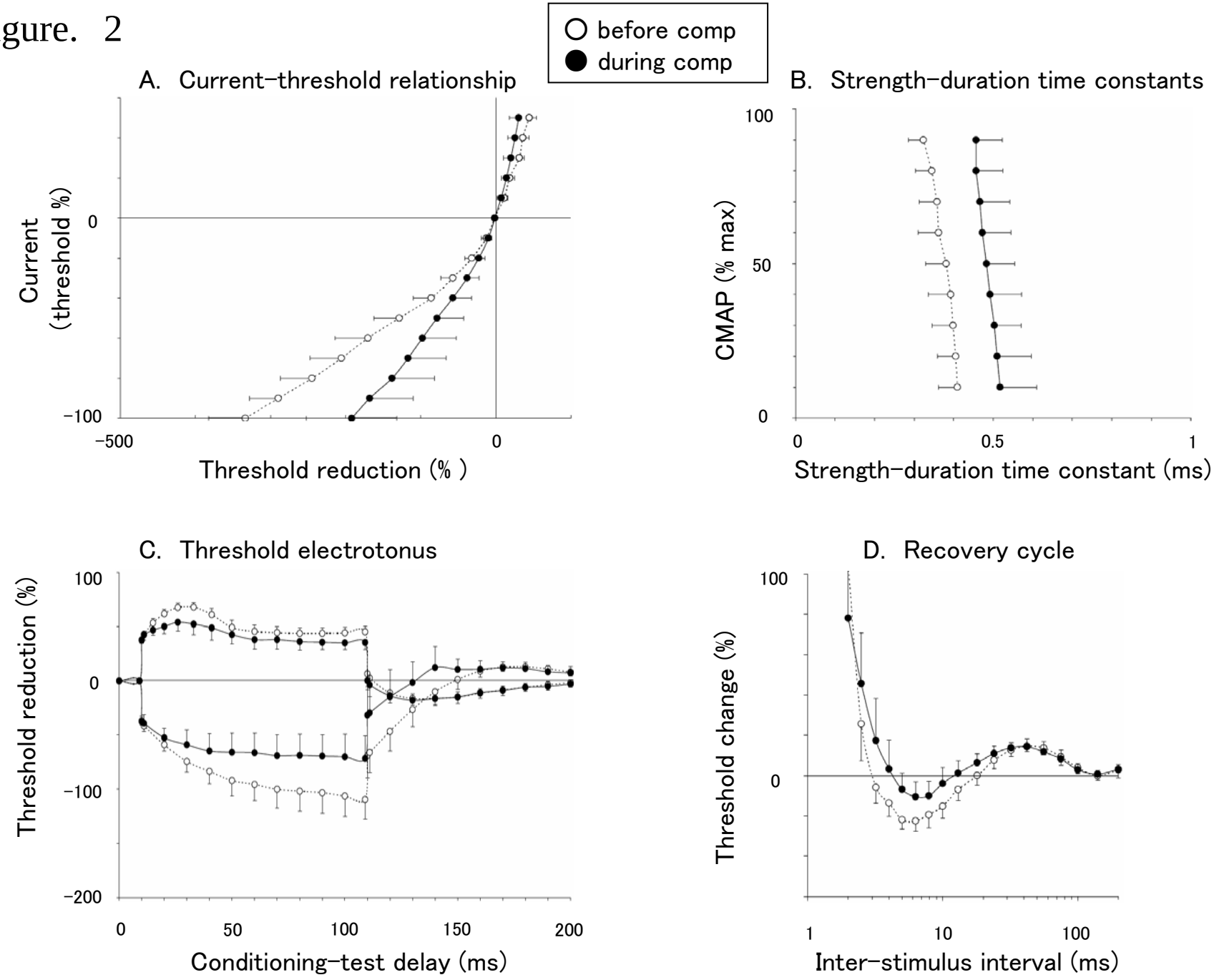
before comp
during comp
A. Current-threshold relationship
Current (threshold %)
0
-100
-500
0
Threshold reduction (%)
B. Strength-duration time constants
CMAP (% max)
100
50
0
0
0.5
1
Strength-duration time constant (ms)
C. Threshold electrotonus
Threshold reduction (%)
100
0
-100
-200
0
50
100
150
200
Conditioning-test delay (ms)
D. Recovery cycle
Threshold change (%)
100
0
1
10
100
Inter-stimulus interval (ms)

Figure. 3

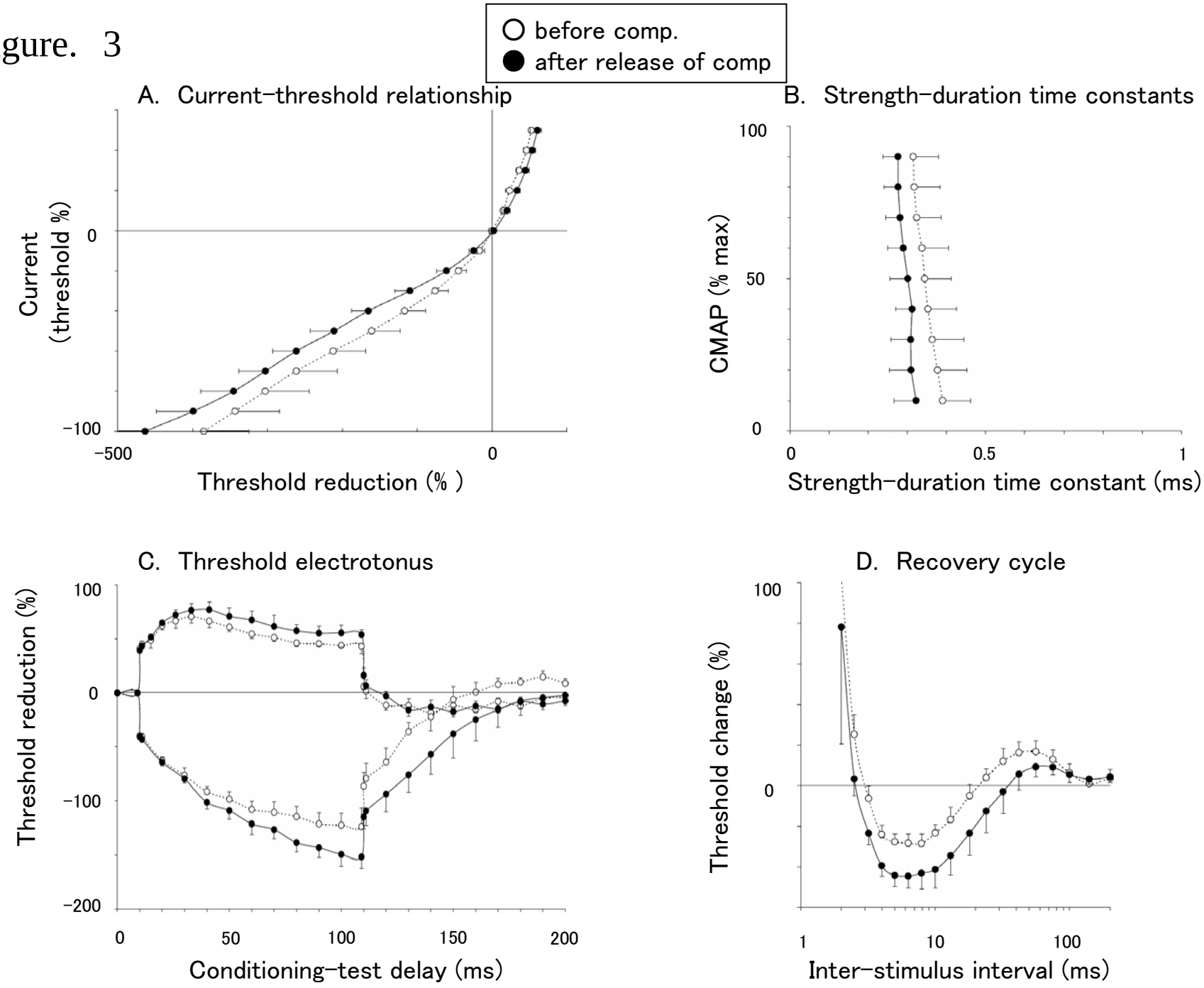
before comp.
after release of comp
A. Current-threshold relationship
Current (threshold %)
0
-100
-500
0
Threshold reduction (%)
B. Strength-duration time constants
CMAP (% max)
100
50
0
0
0.5
1
Strength-duration time constant (ms)
C. Threshold electrotonus
Threshold reduction (%)
100
0
-100
-200
0
50
100
150
200
Conditioning-test delay (ms)
D. Recovery cycle
Threshold change (%)
100
0
1
10
100
Inter-stimulus interval (ms)

Figure. 4

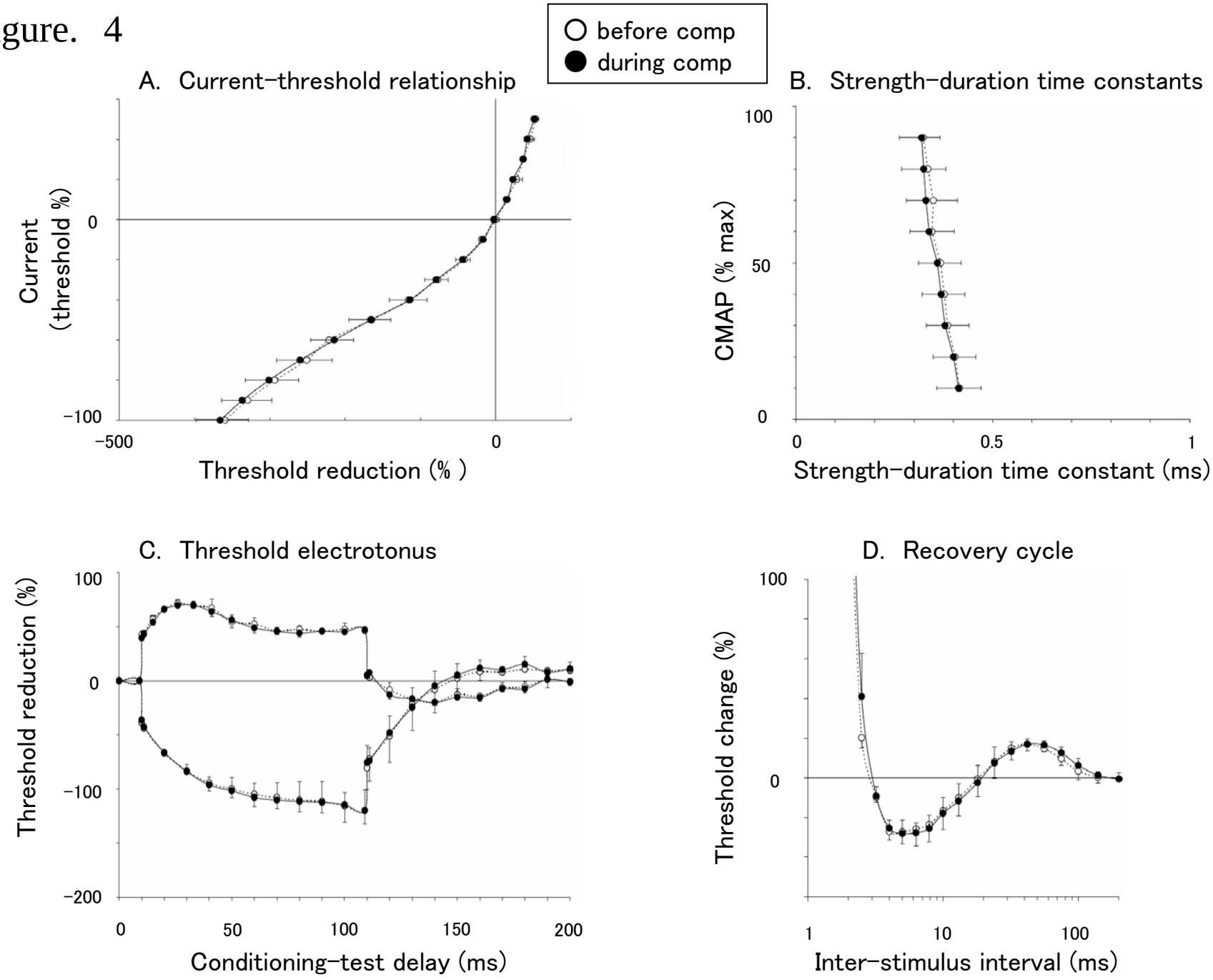
before comp
during comp
A. Current-threshold relationship
Current (threshold %)
0
-100
-500
0
Threshold reduction (%)
B. Strength-duration time constants
CMAP (% max)
100
50
0
0
0.5
1
Strength-duration time constant (ms)
C. Threshold electrotonus
Threshold reduction (%)
100
0
-100
-200
0
50
100
150
200
Conditioning-test delay (ms)
D. Recovery cycle
Threshold change (%)
100
0
1
10
100
Inter-stimulus interval (ms)

Table. 1

Various excitability measurements for median motor axons at the compression site (N=10)

	Before nerve compression	*During nerve compression*	*P-value*
Threshold reduction for			
50% depolarizing currents (%)	44.5±9.3	30.5±12.3	0.007
50% hyperpolarizing currents (%)	-128.5±34.0	-78.2±35.0	0.007
SD time constant (ms) for 40% of max. CMAP	0.39±0.06	0.49±0.08	0.007
TEd [90-100 ms] (%)	43.6±4.8	35.5±6.2	0.007
TEh [90-100 ms] (%)	-104.8±18.7	-69.5±19.9	0.007
RRP (ms)	2.96±0.49	4.97±1.58	0.007
Superexcitability (%)	-26.0±4.2	-10.5±5.4	0.007
Subexcitability (%)	18.7±6.8	15.9±6.3	0.33

Table. 2

Various excitability measurements for median motor axons at the compression site (N=7)

	Before nerve compression	*Immediately after release of compression*	*P-value*
Threshold reduction for			
50% depolarizing currents (%)	52.4±1.7	60.6±5.3	0.028
50% hyperpolarizing currents (%)	-161.1±38.0	-211.1±31.8	0.028
SD time constant (ms) for 40% of max. CMAP	0.35±0.07	0.31±0.04	0.028
TEd [90-100 ms] (%)	44.9±1.4	55.3±5.5	0.028
TEh [90-100 ms] (%)	-120.6±8.9	-146.9±9.6	0.028
RRP (ms)	3.00±0.20	2.77±0.32	0.028
Superexcitability (%)	-28.9±1.5	-45.6±4.8	0.028
Subexcitability (%)	15.9±5.3	10.2±3.7	0.028

Table. 3

Various excitability measurements for median motor axons 2cm distal to the compression site (N=7)

	Before nerve compression	*During nerve compression*	*P-value*
Threshold reduction for			
50% depolarizing currents (%)	52.9±3.8	51.2±3.3	0.67
50% hyperpolarizing currents (%)	-164.6±26.7	-165.8±25.1	0.93
SD time constant (ms) for 40% of max. CMAP	0.38±0.05	0.37±0.05	0.80
TEd [90-100 ms] (%)	46.7±3.8	45.3±1.8	0.35
TEh [90-100 ms] (%)	-113.5±17.5	-113.5±11.0	0.67
RRP (ms)	2.93±0.07	3.01±0.14	0.27
Superexcitability (%)	-28.1±5.9	-28.2±5.6	0.93
Subexcitability (%)	17.0±3.7	17.2±1.9	0.93