Supplementary Methods: Threshold tracking

The threshold-tracking program QTRAC (© Institute of Neurology, Queen Square, London, UK) adjusts the stimulus strength in a feed-back controlled manner for different test paradigms to produce the target response using proportional tracking. We used this technique to investigate the excitability parameters of the median nerve by recording antidromically the sensory nerve action potential (SNAP) as described by Kiernan et al. (Suppl. Figure 5A). Throughout the recording protocol, a stimulus frequency of 1 Hz and a stimulus width of 0.5 ms were chosen unless otherwise stated.

As a first step, a stimulus-response curve was generated. The stimulus strength was gradually stepped up until a maximum response of the sensory nerve action potential was reached (Suppl. Figure 5B-1). An amplitude of ~40% of maximum amplitude was then defined as the target amplitude and the stimulus current needed to reach this amplitude was called ‘threshold current’. This target amplitude is chosen automatically by the program as the point with the maximal slope between 30% and 50% of the stimulus response. The rationale behind the defined ‘threshold current’ is that a small change in stimulus strength would have the largest change in amplitude of the target response. Changes of this ‘threshold current’ were measured continuously in response to various test stimulus configurations and automatically adjusted.

Second, to record the strength-duration relationship, stimuli of different duration (0.1 ms to 0.5 ms) were applied (Suppl. Figure 5B-2). The strength-duration time constant (τSD) was calculated off-line from thresholds measured according to Weiss’s formula.

Third, the recovery of excitability following a single supramaximal 0.5-ms conditioning stimulus was measured (Suppl. Figure 5B-3). Excitability changes were recorded at 18 different conditioning-test intervals decreasing from 200 ms to 1 ms in approximately geometric sequence. The resulting recovery cycle curve allows identification of the phases of excitability changes of a nerve after a supramaximal stimulus (e.g. relative refractory period, superexcitable period, subexcitable period) as described by Raymond (Suppl. Figure 5C). Three stimulus conditions were tested in turn (control test stimulus, supramaximal conditioning stimulus alone, and the combined conditioning and test stimuli) on three different channels. The response to the conditioning stimulus alone subtracted from the response to the combined stimulus results in the effective response to the test stimulus.
Fourth, a threshold electrotonus was recorded (Suppl. Figure 6). The excitability properties of the nerve were altered by passing a polarising 100-ms subthreshold current through the whole nerve. The polarising currents were set to +40% (depolarising) and -40% (hyperpolarising) of the threshold current. Thresholds were then tested at defined delays between 0 ms and 200 ms during and after the start of the polarising current ('threshold-electrotonus'). During this section of the protocol, the control, depolarised and hyperpolarised thresholds were tested in turn on three different channels of the program.

As a last step the current-voltage relationship was recorded (Suppl. Figure 7). Excitability of the nerve was tested at a fixed interval of 200 ms after the start of a polarising current. The polarising currents were stepped down from +50% (depolarising) of the threshold current to -100% (= hyperpolarising current) in steps of 10%. During this section of the protocol, the control and the thresholds after polarisation were tested in turn on two different channels of the program.

References:


37. Weiss G. Sur la possibilité de render comparables entre eux les appareils servant à l’excitation électrique. *Archives of Italian Biology* 1901; 35: 413-47


Suppl Fig 5: Experimental setup and stimulation protocol.

A: Setup for excitability measurements: The median nerve was stimulated with the cathode placed at the wrist and the anode placed on the radial side of the forearm. A sensory nerve action potential (SNAP) was recorded antidromically from Dig. II. The signal was amplified and data were then digitized by a data acquisition unit. According to the signal size recorded a ‘threshold tracking’-software (QTRAC©) adjusted the current applied by the stimulator for each stimulus. If the signal was too small as for the defined response size (defined as 40% of the maximum peak response) the current of the next stimulus was increased and vice versa. Skin temperature was measured at the stimulation site. Oxygen saturation was measured by pulse oxymetry throughout the recording on the same side where the recording was being performed.

B: Graphical depiction of the stimulation patterns used in the protocol. 1: A 0.5 ms rectangular stimulus was steadily increased until the size of the SNAP was maximal. 2: To record the strength duration relationship, stimuli of different widths (0.1 ms to 0.5 ms) were applied 3: After a supramaximal conditioning stimulus, excitability changes were recorded at different conditioning-test intervals between 200 ms and 2 ms in an approximately geometrical sequence. 4: To record a threshold electrotonus, 100 ms subthreshold polarising currents set to +40% (depolarising) and -40% (hyperpolarising) of the target threshold current were applied. Thresholds were tested at defined delays during and after the start of the polarising current. 5: To record the I/V-curve subthreshold currents with different polarisation strengths were applied and threshold was tested at 200 ms after the start of the polarising current.

C: The different periods during a recovery cycle of a sensory afferent nerve are illustrated. The change of currents of the test stimuli to reach the threshold is plotted on the y-axis in a normalised way whereby zero represents the unconditioned control threshold. Immediately after a supramaximal conditioning stimulus the nerve enters the refractory period. The refractory period ends as soon as the curve crosses the zero line for the first time (indicated with the dashed line). Thereafter, the nerve is superexcitable until it becomes subexcitable (second intersection of the curve with the zero line). Normal excitability is restored after 200 ms.

Suppl. Fig. 6. Threshold electrotonus.

A: On the y-axis the reduction of threshold induced by the polarising conditioning current is shown (polarising current starts at 10 ms). Positive values imply that a weaker current was needed to reach
the threshold and vice versa. Threshold changes were unaffected by either propofol or sevoflurane.

**B:** The histograms of the threshold reduction at 100 ms of the conditioning stimulus (indicated by the black arrow in A) illustrate the similarity of the measured values before and after the induction of anaesthesia. Circles represent mean threshold changes at a given time interval. Empty circles indicate values before induction, grey circles represent propofol, black circles sevoflurane after induction. Error bars are SEM.

**Suppl. Fig. 7. Current-threshold relationship.**

**A:** Current-threshold relationship reflects the rectifying properties of the nerve membrane as a response to long polarising conditioning currents. On the depolarising side (right from the zero-axis) the decrease of excitability reflects outward rectification, on the hyperpolarising side (left from the zero-axis) the increase of excitability reflects inward rectification illustrated by the open arrows. **B:** The slope of the current-threshold relationship is the threshold analogue of the conductance. Both, minimum slope of the curve on the hyperpolarising side and resting slope (intersection of the two zero axis) were not altered by propofol and sevoflurane, respectively. Circles represent mean threshold changes at a given polarisation defined as a percentage of the threshold current. Empty circles indicate values before induction, grey circles represent propofol, black circles sevoflurane after induction. Error bars are SEM.
A

**Propofol**
- ○ Before anaesthesia induction
- ● After anaesthesia induction

### Depolarising TE:

- Threshold reduction (%)
- Delay (ms)

**Sevoflurane**
- ○ Before anaesthesia induction
- ● After anaesthesia induction

### Hyperpolarising TE:

- Threshold reduction (%)
- Delay (ms)

B

- TEd(90-100ms) %
- Control
- Propofol
- Sevoflurane

- TE(90-100ms) %
- Control
- Propofol
- Sevoflurane

- P=0.72
- P=0.28
- P=0.37
- P=0.28
- P=0.68
- P=0.66