Supplementary Materials

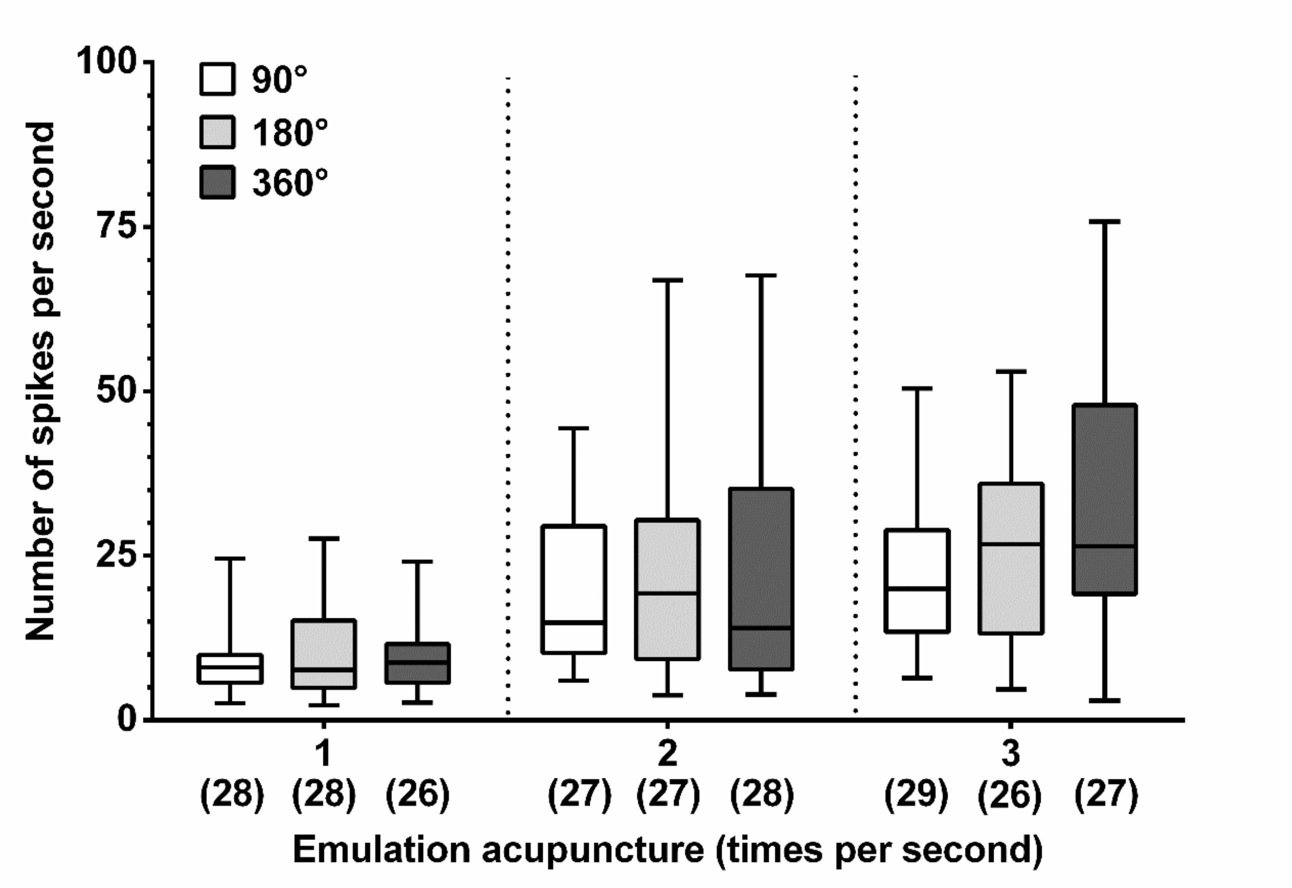

Fig. S1 No significant differences in the numbers of evoked spikes per second between groups with EAc at 90°, 180°, or 360°. Data are expressed as the median and 25–75th percentiles (outliers beyond 1.5 interquartile ranges were removed; non-parametric tests). Digits in the brackets indicate the numbers of single-unit afferent fibers.

1. Factors Affecting Conduction Efficiency of PES at Optimal Frequencies in Afferent Fibers

In the main text, Fig. 5 shows the conduction efficiency of Aδ fibers in response to PES in dense and disperse mode [alternation between 2 and 100 (2/100) Hz] is better than a single frequency (100 Hz) during 30 min. This advantage has three main aspects: (1) higher response ratio in total; (2) lower proportion of fibers with conduction failure; and (3) conduction failure at later times.

In Aδ fibers, the total response ratio was 80.17%, and various conduction failures after 587 ± 177 s occurred in 73% at a single frequency (100 Hz) (Fig. S2A, B). However, conduction failure occurred after 884 ± 221 s among only 33% at 2/100 Hz, and the total response ratio was 97.00% (Fig. S2C, D).

These data indicate that PES at frequencies alternating between 2 and 100 Hz for 3 s each enhances faithful transmission along afferent nerve fibers compared to a single frequency (100 Hz) during relatively long-term stimulation (30 min).

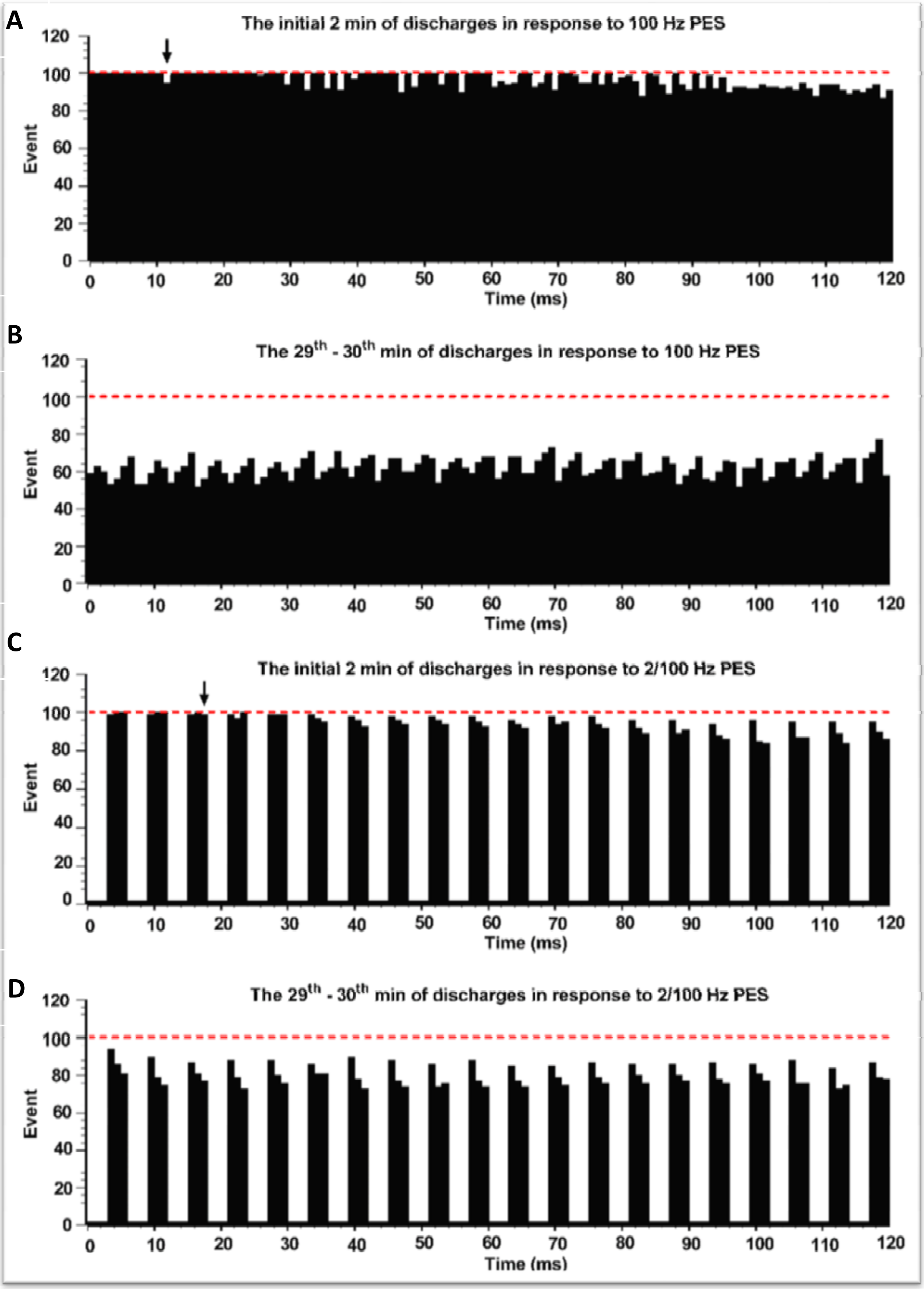

Fig. S2 Example histograms of evoked discharges showing differences in conduction efficiency of PES at 2/100 Hz compared to 100 Hz in Aδ fibers (binwidth 1 s). **A, B** Histograms of the same single-unit afferent fiber (CV 18.63 m/s). Conduction failure occurred at 451 s (arrow), about 60 evoked discharges per second were missed ultimately, and the total response ratio was 79.98% in response to 100 PES. **C, D** Histograms of the same single-unit afferent fiber (CV 22.22 m/s). Conduction failure started at 1095 s (arrow); almost 80 evoked discharges were missed per second in response to dense PES (100 Hz), ultimately in form of staircase deterioration (in a 3-s period of dense stimulation), and the total response ratio was 94.16% with 100% with dispersed stimulation (2 Hz).

2. Patterns of Evoked Discharges in Response to PES at Higher Frequencies (100–1000 Hz)

PES at frequencies <100 Hz was precisely conducted along peripheral afferent fibers during the initial 60 s (Fig. 5B, C). Conduction failure occurred in response to PES at frequencies >100 Hz. In general, occasional conduction failure occurred in response to PES at 200 Hz for Aδ fibers or at 200 Hz and 300 Hz for Aβ fibers, which was characterized as an irregular response (Fig. S3B). The discharges of the Aβ and Aδ fibers in response to higher frequency PES occurred in proportional or burst patterns. A proportional response contained more than one type, such as two- to-one or even four- or five-to-one responses (Fig. S3C, D). Taking the two-to-one response for example, only one of two PES can be effectively transmitted along a peripheral nerve. As for burst responses, the effective within-burst discharge was usually a proportional response (Fig. S3E). Furthermore, the response ratio within a burst decreased gradually with increasing frequency of PES, such as from two-to-one to three- or even four-to-one. Note that Aδ fibers sometimes remained silent for several seconds (no evoked discharges) at 900- or 1000-Hz PES.

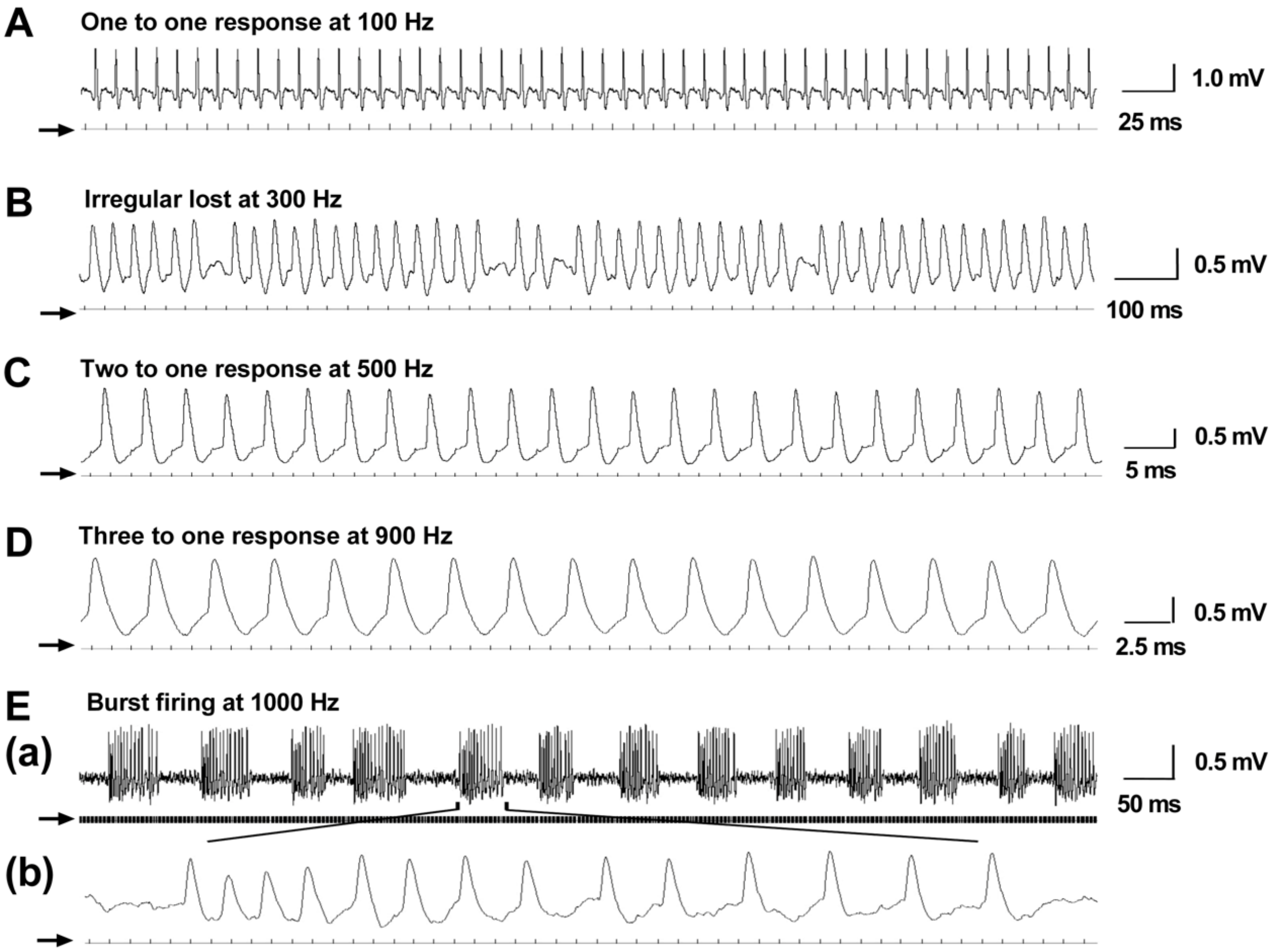

Fig. S3 Continuous recordings showing various types of conduction failure of Aβ fibers in response to PES at various frequencies. **A–E** Conduction velocities were 30.77, 29.80, 30.77, 40.97, and 25.93 m/s, respectively. Fifty PES are shown in **A–D** and Eb for clarity (arrows, PES markers). PES, peripheral electrical stimulation.

3. Threshold of Afferent Fibers in Response to PES at Optimal Frequencies

The number of evoked discharges of afferent fibers induced by electrical stimulation was correlated with both the pulse width and the intensity of stimulation. The threshold intensity of the same fiber increased with decreased pulse width. In other words, the smaller the pulse width, the larger the threshold current.

First, we found that the threshold current of Aβ and Aδ fibers in response to PES at different frequencies (2, 2/15, 15, 2/100, and 100 Hz) corresponded with pulse width. The threshold current of Aβ-fibers in response to PES at 100 Hz and 2/100 Hz (0.61 ± 0.03 and 0.64 ± 0.04 mA) was greater than that in response to 2 Hz, 15 Hz, and 2/15 Hz (0.35 ± 0.02, 0.43 ± 0.02, and 0.43 ± 0.02 mA, respectively) (P <0.001; one-way ANOVA) (Fig. S4A). Similarly, the threshold current of Aδ-fibers in response to 100 Hz (0.51 ± 0.04 mA) was higher than that at the other frequencies noted above (0.53 ± 0.04, 0.51 ± 0.04, 0.72 ± 0.05, and 0.69 ± 0.06 mA, respectively) ($P < 0.05$; one-way ANOVA; Fig. S4B). The threshold current of Aδ-fibers was higher than that of the Aβ-fibers in response to PES at same optimal frequency ($P < 0.05$, and $P < 0.001$; unpaired t test; SFig. 4C).

The results suggested that the thresholds of Aβ and Aδ fibers increase with the frequency of PES as a result of decreased pulse width. Furthermore, a stronger current is required for Aδ fiber activation by PES than for Aβ fibers.

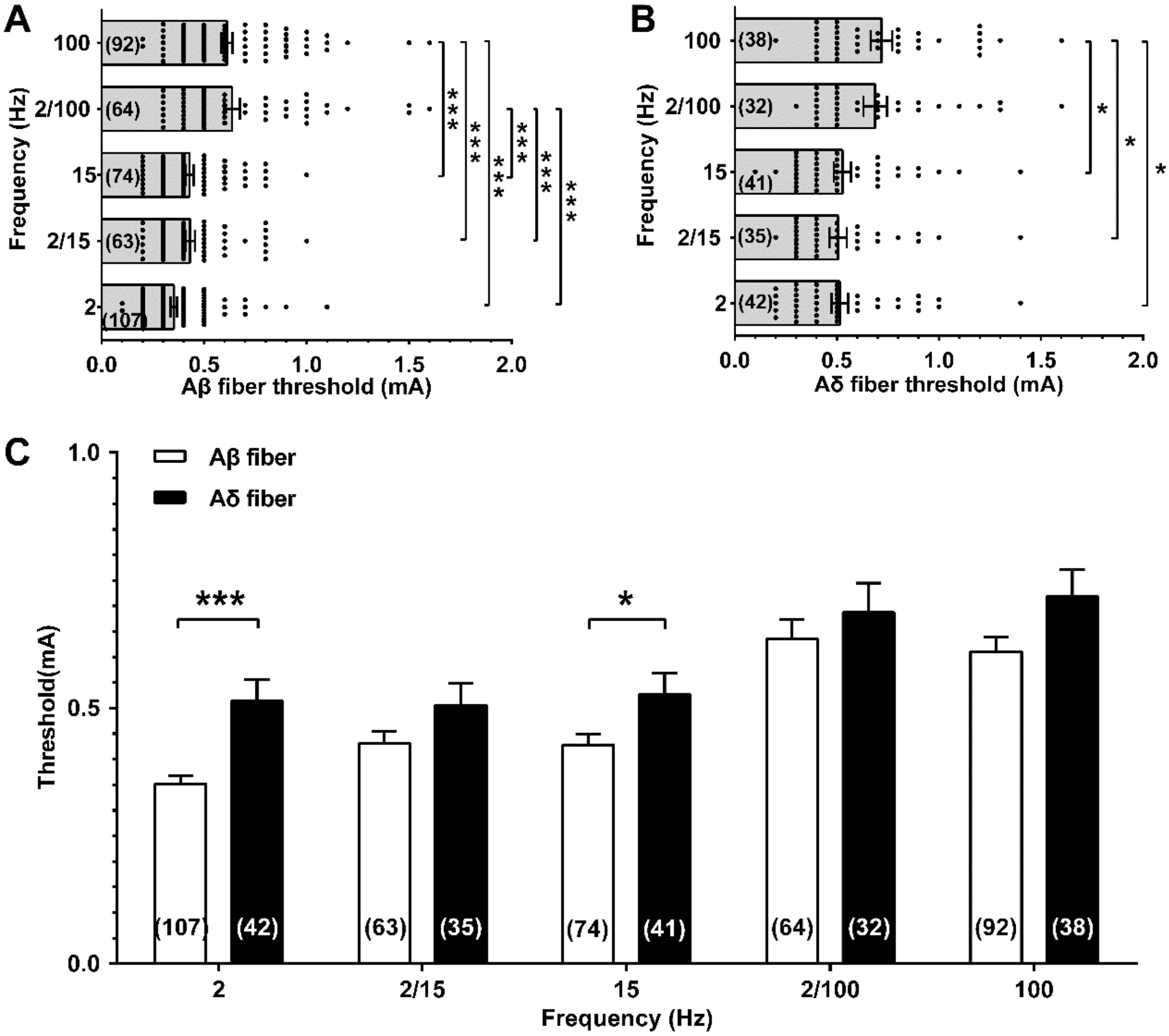

Fig. S4 Thresholds of Aβ and Aδ fibers in response to PES at optimal frequencies. **A, B** The thresholds of Aβ- and Aδ-fibers at various frequencies. Note that the individual dots in scatter diagram overlap and became a thick straight line in some diagrams due to the high density of data. **C** The threshold of Aδ fibers was higher than that of Aβ fibers in response to PES at the same optimal frequency. Digits in brackets represent the numbers of recorded fibers. Data are expressed as the mean ± SEM. $^{*}P < 0.05$, $^{***}P < 0.001$, one-way ANOVA and unpaired *t* test. PES, peripheral electrical stimulation.