Supplementary material

In order to test the duration of the sensitisation phenomenon, two complementary experiments have been performed in parallel to protocol 3 of the printed version. Each protocol for a defined subject was separated from one another by a minimal period of three weeks as in the rest of the experiments, in random order. Following a 2 min period in resting conditions, a total 12 mC current application was delivered through two consecutive 6 mC applications. A first 1 min 0.10 mA anodal current application was performed on the two active probes. Thereafter a second 1 min current delivery was performed on the two active probes for various inter-stimulation intervals. In protocol 3b intervals 10 or 40 min, and intervals were 60 or 90 min in protocol 3c. In each experiment, a recovery period was observed and prolonged until 20 min following the end of the latest second current application. Thereafter, local heating was started simultaneously on the two probes and prolonged for 24 min as previously described.

Results for these complementary experiments are presented with those yet reported in the printed article for 5 and 20 min of inter-stimulation interval. Figure S1 shows the vasodilatation resulting from repeated current application with a 40 min inter-stimulation interval. As noted from the figure, initial one min current application was followed by a slow vasodilatation. This slow vasodilation was prolonged over the whole inter-stimulation period for 5, 10, 20 and 40 min inter-stimulation intervals (S_{LDF} was respectively 0.4 ± 0.5, 0.2 ± 0.5, 0.4 ± 0.6, 0.2 ± 0.5 AU min^{-1}). For the longest (60 and 90 min) intervals between current applications, LDF showed a peak around minute 35 and then past minute 40 slowly decreased. S_{LDF} in the first 35 min following the initial current application was 0.4 ± 0.7 and 0.3 ± 0.4 AU min^{-1} for these two longest experiments. From minute 40 to the initiation of the second current application, for 60 and 90 min inter-stimulation intervals, S_{LDF} was -0.2 ± 0.5 and -0.2 ± 0.5 AU min^{-1} respectively. As a consequence of the slow LDF increases during the first 35–40 min that follow the first current application, Aend was significantly increased compared to LDF_{rest} for 10, 20, 40 and 60 min inter-stimulation intervals (Fig. S2). Due to the decrease observed past the minute 40, Aend was not significantly different from LDF_{rest} for a 90 min interval. Following the second 1 min current application LDF abruptly increased in all cases. S_{LDF} was 8.2 ± 6.2, 7.2 ± 6.4, 5.9 ± 5.4, 2.7 ± 1.9, 2.4 ± 2.3, 0.6 ± 0.7 AU min^{-1} in the first 5 min following the second current application, with maximal value for S_{LDF}: 17.7; 16.3; 15.2; 6.2; 6.0; 1.8 AU min^{-1} for inter-stimulation intervals of, 5, 10, 20, 40, 60, 90 min, respectively. The maximal B_{peak} was observed when the interstimulation interval was 5 min (Fig. 4) and then progressively decreased with the duration of interstimulation interval. No significant change was observed on LDF_{heat} between the different interstimulation interval protocols: 112.4 ± 29.3, 105.5 ± 16.2, 110.9 ± 23.5, 114.6 ± 29.1, 93.7 ± 22.7, 89.9 ± 27.2 for inter-stimulation intervals of, 5, 10, 20, 40, 60, 90 min, respectively

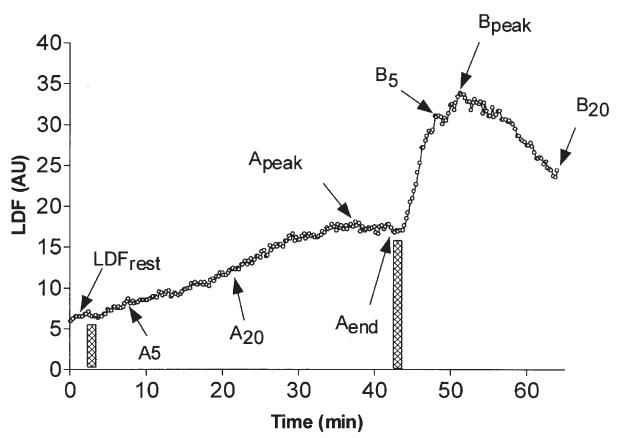

Figure S1. Illustration of the different analysed points on the laser Doppler flowmetry signal (LDF)

Averaged data are presented in arbitrary units, as defined in the section method, during a repeated 1 min, 0.10 mA anodal current application (bars), with 40 min inter-stimulation interval. The heating period is not presented to simplify the figure .

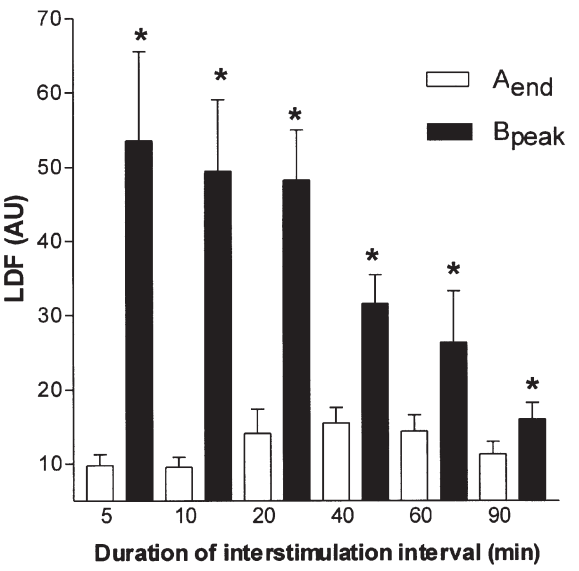

Figure S2. Laser Doppler flowmetry (LDF) values observed for different intervals between two consecutive 1 min 0.10 mA anodal current application

The data are means ± s.e.m. White bars is LDF before the second current application (A_{end}), Black bars is peak LDF following the second current application (Bpeak). All values are significantly increased from rest. (* $P < 0.05$ B_{peak} *vs.* A_{end}.)

DISCUSSION

It is of interest to note that, in parallel to what is reported in the printed paper, in our experimental conditions, the time courses for sensitisation to current resulting in an abrupt ample vasodilatation, and for the slow vascular response resulting from a single current application, are different. The maximal amplification of the vascular response to anodal current occurs during the first 10 min following the initial current delivery and the resulting vasodilatation reaches a maximum within 5 min, whereas the slow vasodilatation of limited amplitude occurring following a single current application reaches a maximum in about 35–40 min.

As shown from time course analysis of the two phenomena, the magnitude of these changes in the local physiological conditions following isolated anodal current application is not proportional to the non-specific apparent vascular response for a defined delay following the first current delivery as could be recorded from a control probe. This further confirms that the sole subtraction of the apparent vascular effect of the current may not exactly estimate this effect. Finally, sensitisation lasts for about one hour.