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Selective Stimulation of C Fibers by an Intra-Epidermal Needle Electrode in Humans

Naofumi Otsuru [1,2,*], Koji Inui[1], Koya Yamashiro [1,2], Takahiro Miyazaki [1,2], Ichiro Ohsawa [3], Yasuyuki Takeshima [1], and Ryusuke Kakigi [1,2]

[1] *Department of Integrative Physiology, National Institute for Physiological Sciences, Okazaki 444-8585, Japan*

[2] *Department of Physiological Sciences, School of Life Sciences, Graduate University for Advanced Studies, Hayama, Kanagawa, Japan*

[3] *The first Department of Surgery, Mie University School of Medicine, Tsu 514-8507, Japan*

Abstract: We recorded evoked potentials (EPs) induced by intra-epidermal electrical stimulation using a needle electrode with specific parameters. We identified the fibers activated by this specific stimulation by assessing the conduction velocity (CV) of the peripheral nerve. The EPs were recorded from the Cz electrode (vertex) of the International 10-20 system in ten healthy male subjects. The dorsum of the left hand and forearm were stimulated with an intensity of 0.01 mA above the sensory threshold. The mean P1 latency of EPs for the hand and forearm were 1007 ± 88 and 783 ± 80 ms, respectively, and the CV estimated from the latency of P1 was 1.5 ± 0.7 m/s. The CV indicated that the fibers activated by the stimulation were C fibers. Since the method of stimulation is convenient and non-invasive, it should be useful for investigating the functions of small fibers.

INTRODUCTION

In studies of sensory systems, a well-controlled stimulus is required to activate the system being examined. An experimental stimulus should be quantifiable, and reproducible (regularity of intensity and time distribution). Additionally, for clinical application, safety, low cost, and simplicity of use are required. Regarding the nociceptive system, no method of stimulation fulfilling these requirements is available, a technical drawback that has prevented progress within this field. The selective activation of C fibers with little or no concurrent activation of other sensory modalities is particularly difficult.

Various techniques (for review, see [1]) have been used to investigate C fiber-related cerebral processing [2-8] as well as conduction velocity (CV) [9], each with its own strengths and weaknesses.

The most common way of activating C fibers is to use a laser to stimulate a tiny area of skin [10]. Lasers can stimulate C fibers specifically with minimal effects on other fibers, but are expensive and hard to control. Electrical stimulation is also useful for investigating the nociceptive system, since the equipment is easier to use and the method itself is non-invasive. Despite its technical advantages, however, conventional electrical stimulation activates thicker fibers at a lower current intensity than C fibers.

We have developed a method of intra-epidermal electrical stimulation (IES) for the selective activation of Aδ fibers [11-16]. Since this method is easy to control, does not require special equipment, and provides a steep rise of stimulation, it would be good for studying the C fiber nociceptive system if it could activate C fibers selectively. We noticed in previous studies that IES actually activates C fibers when a stimulus intensity higher than the threshold for Aδ fibers is used. Similar results were reported by Nilsson *et al.* [17] and Nilsson and Schouenborg [18] who used a needle-like electrode. However, this indicates that the stimulus inevitably activates Aδ fibers at the intensity necessary to activate C fibers. Here we report that IES can activate cutaneous C fibers selectively when specific parameters are employed.

METHODS

Subjects

The experiments were performed on ten healthy male volunteers (25-43 years). The study was approved in advance by the Ethics Committee of the National Institute for Physiological Sciences, Okazaki, Japan, and written consent was obtained from all the subjects.

Stimulation

We used a method of intra-epidermal stimulation (IES) developed in our laboratory for the selective activation of Aδ fibers [12]. For IES, we used a concentric bipolar needle electrode (Nihon Kohden, Tokyo, Japan) which consisted of an outer ring 1.2 mm in diameter and an inner needle that protruded 0.1 mm from the outer ring. For the selective stimulation of C-fibers, the following parameters were used: 1) The anode was the inner needle and the cathode was the outer ring. 2) The electric pulse was a triangular wave with a rise and fall time of 1 ms. 3) The stimulation was a train of

*Address correspondence to this author at the Department of Integrative Physiology, National Institute for Physiological Sciences, Okazaki 444-8585, Japan; Tel: +81 564 55 7811; Fax: +81 564 52 7913;
E-mail: ootsuru@nips.ac.jp

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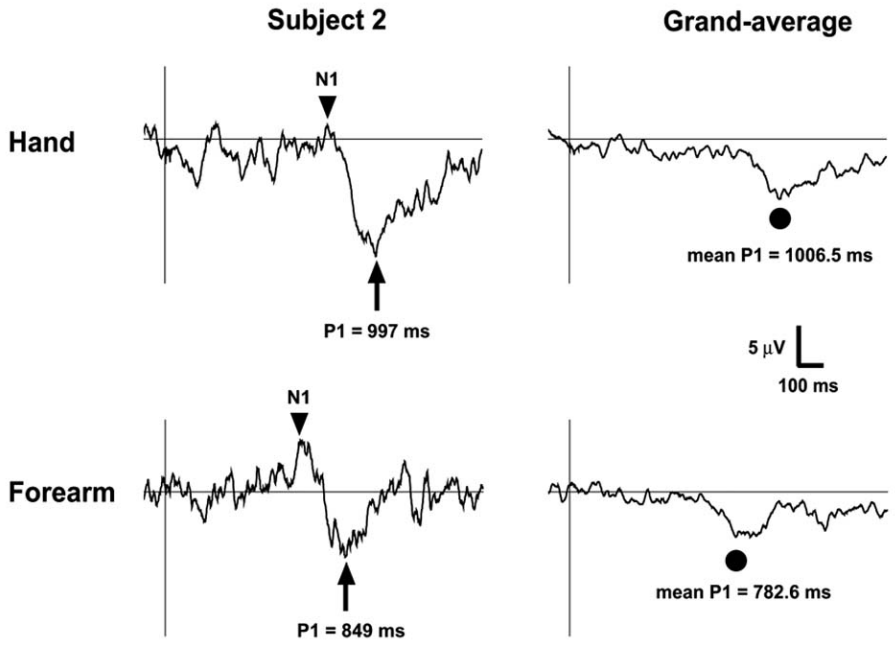

Fig. (1). Evoked potentials following intra-epidermal electrical stimulation recorded at Cz. Waveforms of evoked potentials in a representative subject (left) and grand-averaged waveforms (right). Arrowheads and arrows indicate the peak latency of the negativity and positivity, respectively. Circles in the right traces indicate the mean peak latency.

three pulses with an interstimulus interval (ISI) of 20 ms. 4) Three electrodes 10 mm apart were used for augmentation of the response. These parameters were determined based on results of preliminary experiments showing that 1) the standard cathodic stimulation always activated Aδ fibers at a lower intensity than that for C fibers, 2) a single pulse of anodal current rarely elicited C-fiber-related brain potentials or sensations while a train of 3-5 pulses was very effective at augmenting the response of C fibers, 3) thicker fibers seemed to prefer a steeper rise in the pulse, 4) a train of pulses with an ISI shorter than 20 ms more effectively augmented the activation of thicker fibers than C fibers, and 5) multiple electrodes were sometimes useful to elicit clear sensations due to the activation of C fibers. These results are generally consistent with the findings that 1) an anodal current is theoretically effective at stimulating cutaneous fibers running vertical to the skin's surface [19] such as branches of C-fibers innervating the epidermis [20, 21], 2) a pulse of longer duration is necessary to stimulate thinner fibers [22] and 3) a substantial spatial and temporal summation of C-fiber impulses is required to produce painful sensations [23, 24].

The electrical stimulus was applied to the dorsum of the left hand and forearm. The stimulation was started at with an intensity of 0.01 mA and increased in steps of 0.01 mA until the subject felt a sensation (threshold). Subjects were instructed to press a button quickly when they felt any sensation and the reaction time (RT) was measured. The mean sensory threshold was 0.04 ± 0.01 and 0.04 ± 0.01 mA at the hand and forearm, respectively. After confirming that the RT was in the range of C fiber transmission (700-1500ms), we recorded evoked potentials (EPs) following IES at an intensity 0.01 mA above the sensory threshold. At this intensity, single or double pulses did not elicit any sensations or EPs.

Recording of Evoked Potentials

EEG signals were recorded from the Cz electrode referenced to the linked earlobes (A1-A2) of the International 10-20 system. A pair of electrodes placed on the supra- and infra-orbit of the right eye was used for recording electro-oculograms. The impedance of the electrodes was kept below 5 kΩ. The EEG signals were recorded with a bandpass filter of 0.1-100 Hz at a sampling rate of 1000 Hz. The window of analysis was from 100 ms before to 1500 ms after the stimulus onset. The 100-ms period before the stimulus was used as the DC baseline. Since the sensation elicited by the stimulation was relatively weak, the subject was asked to attend the stimulus. At least ten artifact-free responses were collected and averaged for each stimulation site.

The peripheral CV was calculated by dividing the difference in peak latency between the EP responses following the hand and forearm stimulation, by the distance between the two sites. Data were expressed as the mean ± SD.

RESULTS

The stimulation did not elicit a C fiber-related sensation or EPs in two of ten subjects. Therefore, EP data obtained from eight subjects were used for the analysis. The sensations produced by IES were a weak painful sensation described as "flicking", "burning", or "long-lasting weak pricking" (n = 4), and a light touch sensation described as a "faint touch" or "light pressure" (n = 4).

Representative EP waveforms of a single subject and the group-averaged waveforms are shown in Fig. (**1**). Similar to previous studies using laser stimulation [25], the stimulation evoked a negativity followed by a positivity (P1). However in some subjects, the negativity was unclear and its peak

Table 1. Peak Latency of EPs and CV

Subject	Peak latency of P1 (ms)		Distance (cm)	CV (m/s)
	Hand	Forearm		
1	992	717	24.3	0.9
2	997	849	27.4	1.9
3	991	650	28.8	0.8
4	955	792	26.2	1.6
5	1092	881	25.2	1.2
6	887	781	24.9	2.4
7	966	862	25.4	2.4
8	1172	729	25.6	0.6
mean	1006.5	782.6	25.9	1.5
SD	87.7	80.4	1.5	0.7

latency was difficult to identify. Thus, we used P1 for the analysis, which was large in amplitude and detected in all eight subjects. The mean P1 latency following hand and forearm stimulation was 1007 ms and 783 ms, respectively (Table **1**). The peripheral CV calculated from the latency difference of P1 was 1.5 ± 0.7 m/s.

DISCUSSION

The stimulation did not elicit a C fiber-related sensation or EPs in two of ten subjects. This might be due to individual difference in thickness of the corneum. The electrode we used was consisted of an inner needle that protruded 0.1 mm from the outer ring and we used a very weak current for selective stimulation of C-fibers. Therefore, in subjects with the thicker corneum, it is possible that the weak current could not reach to the epidermal area in which free nerve endings are located. Our previous study using a laser beam which is the most common way for activating C fibers also failed to elicit C fiber-related responses in four out of 17 subjects [26].

In the present study, P1 peaked at around 800 ms for the forearm and 1000 ms for the hand stimulation. In previous studies using a laser beam to a tiny area of skin [4, 9, 10, 27], which is known to selectively activate C fibers, the mean P1 latency for stimulation of the hand was 930-1144 ms, which is very similar to the present results. The estimated CV of 1.5 m/s in this study was also consistent with previous studies showing a value of 0.8 – 2.8 m/s by averaged EEG [4, 27, 28] and 0.4 – 1.8 m/s [29] by microneurography. In terms of the precise measurement of CV, microneurography is superior to EEG. However, since C-fibers have a very slow CV, the latency difference between the two stimulation sites was enough long (224ms) to correctly detect by EEG. Another weakness of the present method is that we could not know receptive properties of C fibers unlike microneurography (for review, see [30]).

In addition, we also considered that our method mainly stimulated C fibers, since C fiber-related EPs do not appear on the concomitant activation of other fibers. For example, laser stimulation that activates both Aδ and C fibers elicits only the Aδ components without responses at latencies compatible with ultra-late components related to C fibers [31-33]. Likewise, C fiber-related cortical responses are only recorded when the concomitant activation of Aδ fibers is suppressed, *i.e.* a pressure nerve block [34] or heating the skin below the Aδ threshold [4]. In the present study, since there was no consistent response at a latency earlier than 400 ms, the results suggested a selective activation of C fibers by our method.

Laser beams applied to a tiny area [4, 7, 9, 28, 31, 35] have been used to selectively activate C fibers, which is considered difficult to do with electrical stimulation, since C fibers generally have a high threshold to electrical stimulation. However, the present results indicate that this threshold is not so high when appropriate parameters are chosen. In support of this notion, unmyelinated afferents respond to skin deformation in mammals [36-38], and have a low mechanical threshold in human skin [39]. In addition, recent studies suggested that low-threshold C mechano-receptive afferents provide information about pleasant touch [40-42], although their functional role remains unclear. In the present study, we used a very weak current (about 0.05 mA) and half of the subjects felt the evoked sensation as a light touch. Therefore, it is possible that our method preferentially activates the low-threshold C fibers.

In conclusion, intra-epidermal electrical stimulation successfully activated cutaneous C fibers selectively. Because the method is easy to control and non-invasive, it should be useful for investigating the functions of small fibers both for basic research and for clinical examinations, although the parameters remain to be refined for a more consistent and stronger stimulation of C fibers, for example, by changing the waveform of the pulse or duration of the stimulus [43-45].

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