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Neurophysiological evidence for spatial summation in the CNS from unmyelinated afferent fibers

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Activation of the central nervous system by unmyelinated afferent fibers is known to require temporal summation[1,2]. However, the characteristics of spatial summation have not been clearly described. In the present study the possibility of spatial summation in the central nervous system of activity from unmyelinated afferent fibers is tested using the somatosympathetic reflex.

A short latency sympathetic response can be elicited following the stimulation of peripheral myelinated afferent nerve fibers (somatosympathetic A reflex). However, if unmyelinated C fibers are also stimulated, an additional long latency response is present (somatosympathetic C reflex)[7,11]. Because of the different latencies of the somatosympathetic A and C reflexes, the somatosympathetic C reflex can be distinguished and used to study the central nervous system responses to unmyelinated afferent nerve stimulation. The somatosympathetic C reflex requires temporal summation to be elicited[11]. That is, a short train of pulses must be delivered to the unmyelinated afferent fibers to elicit the C reflex. If the somatosympathetic C reflex can also be elicited by single pulses to the several different nerves instead of several pulses to one nerve, this would provide evidence for spatial summation in the central nervous system of activity from unmyelinated afferent fibers.

Eight adult cats (2.5–4.5 kg) were anesthetized with α-chloralose (56 mg/kg, i.v.) after cannulation of the femoral vein under chloroform preanesthesia. Bilateral vagotomies were performed, and carotid sinus nerves were severed bilaterally to prevent blood pressure-induced changes in sympathetic nerve activity. Following tracheotomy, animals were artificially ventilated and paralyzed with gallamine triethiode (4 mg/kg). The sympathetic nerve activity was recorded from the cervical sympathetic trunk while stimulating the afferent nerves in the hindlimb. The cervical sympathetic trunk was cut just central (caudal) to the superior cervical ganglion and freed from the surrounding tissue. Evoked compound action potentials were recorded from the whole nerve prep-

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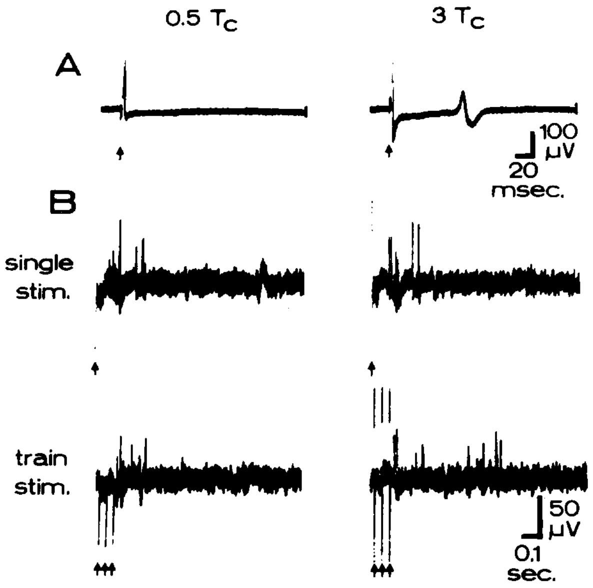

Fig. 1. A: compound action potentials of the peroneal nerve. Stimulus intensity of 0.5 T_c (half the C fiber threshold) elicited only an A fiber volley while stimulus intensities 3 T_c (3 times the C fiber threshold) produced an additional, delayed C fiber volley (conduction velocity, 0.9 m/sec). Each recording consists of 5 superimposed responses. Arrows indicate stimulation. B: somatosympathetic reflexes recorded from a few-fiber preparation of the cervical sympathetic trunk during single and train stimulation of the peroneal nerve with intensities sub- and suprathreshold to C fibers. Note that the long latency C reflex is present only when a train of pulses was delivered with suprathreshold intensities to C fibers. Arrows indicate stimulation. T_c, activation threshold for C fibers.

aration in 4 cats. The sympathetic preganglionic nerve potentials were recorded by a pair of stainless steel electrodes and amplified with an A–C amplifier (half amplitude frequencies set at 0.1 Hz and 2 KHz). The amplified signal was averaged with a data retrieval computer (Nuclear Chicago 7100). Activities of 6 few-fiber sympathetic preganglionic preparations were recorded from the remaining 4 cats. The cervical sympathetic trunk was divided carefully under the dissecting microscope until one or two spontaneously active units were found. The unit activity was also recorded by a pair of stainless steel electrodes and amplified with the A–C amplifier (half amplitude frequencies set at 10 Hz and 2 KHz). The amplified signals were fed into a window discriminator output pulses by a PDP-12 computer. The common peroneal, tibial and sural nerves of the both hindlimbs were cut and prepared for stimulation. The central end of the cut peripheral nerves were stimulated with an intensity which was suprathreshold to the unmyelinated afferent fibers and with a frequency of 0.3 Hz. Warm mineral oil pools were made around all exposed nerves. The rectal temperature of the animal was maintained at 37 $\pm$ 1 °C by a heating pad.

The afferent volleys recorded from the distal branches of the peroneal nerve were evoked by stimulation of the more proximal portions. Fig. 1A shows evoked compound action potentials from stimulation with intensities sub- and suprathreshold to C fibers.

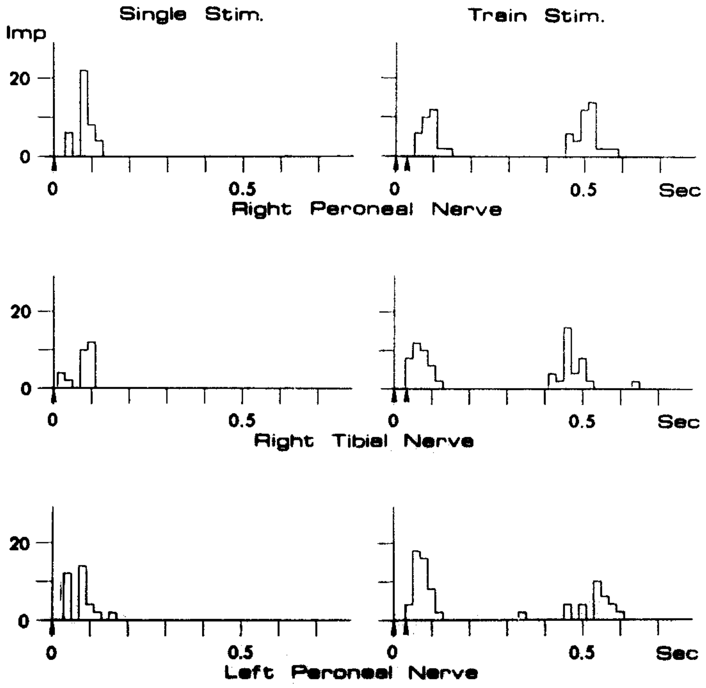

Fig. 2. Poststimulus–time histograms showing the temporal summation of the somatosympathetic C reflex. Three nerves were stimulated individually with the frequency of 0.3 Hz and intensity of 4 T_c (4 times the C fiber activation threshold). Sympathetic unit activity was recorded from the cervical sympathetic trunk. Poststimulus–time histograms were formed after 40 successive stimulations. Note that it required a train of pulses to elicit the late C reflex component for all 3 nerves. Arrows indicate stimulation. Imp, number of impulses per bin (20 msec).

Fig. 1B shows the somatosympathetic A and C reflexes elicited by stimulation (0.3 Hz) of the peroneal nerve with various combinations of stimulus parameters. Single shock stimulation with an intensity suprathreshold to C fibers elicited only a sympathetic A reflex. When 3 pulses were applied in trains with interspike interval of 30 msec, an additional longer delayed C reflex component appeared. This sympathetic C reflex disappeared if the stimulus strength was lowered below the threshold of the afferent C fibers. These results confirm the notion that temporal summation is required to elicit the somatosympathetic C reflex.

Fig. 2 shows poststimulus–time histograms from another sympathetic unit during the stimulations of right peroneal, right tibial and left peroneal nerves with intensities suprathreshold to the C fibers. For all 3 nerves, single shock stimulation elicited only the somatosympathetic A reflex. When two pulses were given in train with 30 msec interspike interval, and additional late C-reflex component appeared. Therefore, the somatosympathetic C reflex from all 3 nerves required temporal summation.

Instead of a train of pulses to one nerve, single pulses were delivered in succession to two different nerves while recording from the same sympathetic unit. Fig. 3A shows poststimulus–time histograms of the unit during stimulation of the right peroneal and right tibial nerves. A single pulse was delivered to the right tibial nerve 70 msec after

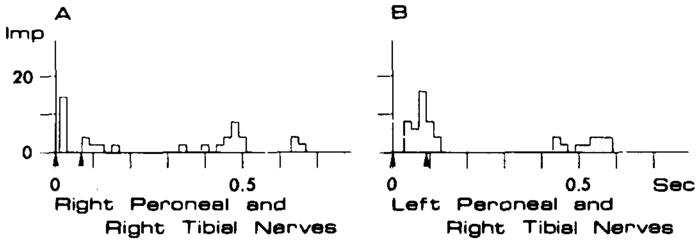

Fig. 3. Poststimulus–time histograms showing the spatial summation of the somatosympathetic C reflex. Single pulses were delivered to two unilateral (A) or bilateral (B) hindlimb nerves in succession while recording from the same sympathetic preparation as in Fig. 2. Stimulus frequency was 0.3 Hz and the intensity was 4 T_c. Poststimulus–time histograms were formed after 40 successive stimulations. Arrows indicate stimulation. Imp, number of impulses per bin (20 msec).

a single pulse stimulation of the right peroneal nerve. As shown in Fig. 3A, this combination of stimulations also elicited the somatosympathetic C reflex. A single pulse to each nerve individually never elicited the sympathetic C reflex as indicated in Fig. 2. The data in Fig. 3A suggest that the spatial summation from unilateral unmyelinated afferent fibers can activate the central nervous system. Fig. 3B shows the somatosympathetic C reflex when a single pulse was delivered to the right tibial nerve 90 msec after a single pulse to the left peroneal nerve. The sympathetic C reflex elicited by the stimulus combination of right and left hindlimb nerves suggests that the central nervous system can also be activated by spatial summation from bilateral unmyelinated afferent inputs.

Temporal and spatial summations were consistently observed both with the compound action potential recording from the cervical sympathetic trunk in 4 cats and with 6 few-fiber preparation recordings in another 4 cats. In most preparations, however, a train of at least 3 pulses was usually required to elicit temporal summation of the somatosympathetic C reflex. When it required 3 pulses for temporal summation, it was necessary to stimulate 3 different nerves with single pulses to elicit spatial summation.

The somatosympathetic C reflex is known to demonstrate a recruitment phenomenon[11]. That is, the size of the reflex progressively increases with repeated stimuli to unmyelinated afferent fibers. The recruitment phenomenon was observed not only during temporal but also during spatial summation. The poststimulus–time histograms shown in all figures of the present experiment started to form after 20 stimuli which was beyond the period required for development of the maximal steady response.

When the somatosympathetic C reflex was elicited by spatial summation, the sequence and time delays between the single pulses to different nerves had to be carefully selected. If the timing was off more than 30 msec, the sympathetic C reflex could not be elicited. This separation of stimuli to different nerves was generally equivalent to the difference in latency of the sympathetic C reflex during temporal summation for each nerve. As indicated in Fig. 2, the C reflex latency was shortest for the right tibial nerve but was longest for the left peroneal nerve in this particular preparation. This is the reason why the sequence and time separation between stimulation of the two nerves were chosen to elicit the C reflex shown in Fig. 3. This property may be due to the different size distribution of unmyelinated fibers in different nerves since the difference in latency between the A and C reflexes is due to the peripheral afferent conduction delay[11].

The somatosympathetic C reflex in Fig. 2 is larger than the one in Fig. 3. This observation suggests that the temporal summation is more effective than the spatial summation for eliciting an evoked somatosympathetic C reflex. Another explanation is that the timing of the stimulation during the spatial summation in Fig. 3 may not be optimal for the summation. However, the responses evoked by spatial summation were never observed to be larger than those evoked by temporal summation no matter what sequence or interstimulus interval was used. This suggests that the temporal summation is more effective than the spatial summation, at least for the somatosympathetic reflex.

The sensations of warmth and some pain sensation are mediated by unmyelinated afferent fibers[3,6]. The sensation of warmth has characteristics of both temporal and spatial summation[5,8,9]. Furthermore, the warmth sensation can spatially summate by bilateral inputs. Marks et al. indirectly suggested that the site of summation may be the central nervous system[10]. The sensation of pain also shows both strong temporal[3] and small but significant spatial summation[4]. Therefore, the present study agrees with these psychophysical experiments and shows direct neurophysiological evidence for the temporal and spatial summation in the central nervous system of activity from unmyelinated afferent fibers.

The exact structures in the central nervous system which are responsible for the temporal and spatial summation are unknown. The fact that bilateral inputs can result in spatial summation in the central nervous system may suggest that structures beyond the secondary neuron are responsible for the spatial summation.

It is concluded that the central nervous system can be activated by spatial summation of activity from unmyelinated afferent fibers as shown in the somatosympathetic C reflex. This spatial summation can be observed with bilateral afferent inputs and displays a recruitment phenomenon.

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