# A Neurophysiological Study on the Sympathetic Premotor Nuclei in the Pons and Medulla Oblongata

by *Yasuhiko Kira [1]* Taku Ogura [2], Yasuo Mikami [2],Shunzo Aramaki [1],Fumihiko Nakanishi [1],Toshikazu Kubo [2]*

[1] Department of Orthopedic Surgery, Kyoto Prefectural Rehabilitation Hospital for the Mentally and Physically Disabled, Kyoto, Japan

[2] Department of Orthopedic Surgery, Kyoto Prefectural University of Medicine, Kyoto, Japan

***Summary***

The aim of this study was to neurophysiologically demonstrate the activities of the premotor nuclei of sympathetic vasomotion, by capturing the diachronic changes in the action potentials which are generated in the pons and medulla oblongata. To do so, ten male Wistar rats weighing 300g were used as subjects. Microelectrodes were inserted in the muscular branch of the sciatic nerve and the ventral side of the pons and medulla oblongata, and the muscle sympathetic nerve activity (MSA) was induced. The regular spontaneous action potentials, which synchronize with muscle sympathetic nerve activity, were observed in the rostral ventrolateral medulla oblongata (RVLM), and the differences among the action potentials of individual cells of the RVLM region noted. Autonomic postganglionic nerves are controlled in turn by preganglionic nerves that originate from specific nuclei in the medulla. These nerves directly influence cardiovascular function by regulating the rate and force of contraction of the heart and the diameter of blood vessels. RVLM cells in fact exert a widespread control over the sympathetic outflow. We conclude from the experiment that premotor nuclei of sympathetic vasomotion exist in the RVLM.

***Introduction***

It is thought that the postganglionic sympathetic fibers are controlled by preganglionic fibers which originate from particular nuclei in the medulla oblongata, thoracic spinal cord and upper lumbar spinal cord, have a local autoregulatory mechanism, and have direct effects on the functions of the cardiovascular system as well as on the circulation of hormones.

Recently, researches on the anatomical *(Strack et al., 1988),* functional *(Janig, 1985),* and chemical *(Krukoff, 1985)* characteristics of the preganglionic fibers and synaptic inputs have become well advanced. Therefore, we assume that by capturing the activation pattern and response to stimulations of the premotor nuclei which regulate the sympathetic activities that are difficult to capture, and by comparing these to other vital rhythms, it may become possible to reveal the complicated mechanism of the sympathetic activities, which in turn will be useful in the treatment of diseases including functional disorders of the sympathetic nervous system.

The activities of the human postganglionic fibers of the peripheral sympathetic nerves consist of skin sympathetic nerve activity (SSA) *(Burke et al., 1977; Vallbo et al., 1979; Wallin and Eckberg, 1982),* which controls the sweat glands and cutaneous vasomotion, and MSA *(Hagbarth et al., 1975; Yatomi et al., 1989),* which controls the vascular smooth muscles in the skeletal muscles. It is possible to separately record SSA and MSA by using microneurography *(Hagbarth et al.,1972)*. In the present study, by evaluating the interrelationship between MSA and the diachronic changes in

**Correspondence:* Yasuhiko Kira, Department of Orthopedic Surgery, Kyoto Prefectural Rehabilitation Hospital for the Mentally and Physically Disabled, Naka Ashihara, Johyo City, Kyoto 610-0113, Japan.
Tel: +81-774-54-1400. Fax: +81-774-54-3616.
E-mail: kira-jscn@umin.ac.jp

the action potentials which are generated in the pons and medulla oblongata, we demonstrated the existence of the sympathetic premotor nuclei which generate the action potentials of the vasomotor fibers of the sympathetic nervous system.

***Materials and Methods***

The study subjects were 10 male Wistar rats, each weighing approximately 300 g. This study was approved in accordance with the ethical code for conducting animal experiments which was established by Kyoto Prefectural University of Medicine (approval number: M101).

Firstly, the room temperature was maintained at 26-28°C and, after anesthesia was induced using pentobarbital sodium (25 mg/kg), the rats were fixed on a stereotactic table. The right sciatic nerve was exposed, and a tungsten microelectrode with an apical diameter of 1 μm and an impedance of 3 to 5 MΩ (Unique Medical Co., UJ3002B, Tokyo, Japan) of the type below was inserted into the muscular branch of the sciatic nerve and fixed to this site. Thereafter, using an amplifier with a frequency range of 500 to 5 kHz (Nihon Kohden Co., MEM4104, Tokyo, Japan), the action potentials of MSA were induced.

Secondly, the pons and medulla oblongata were exposed. A tungsten multimicroelectrode, made by accumulating microelectrodes with an apical diameter of 1 μm and an impedance of 3-5 MΩ, and with which it was possible to simultaneously derive 28 potentials (Unique Medical Co., PS99006, Tokyo, Japan) (Fig. 1A), was inserted from the dorsal side of the transition between the head and neck, and was fixed to a depth of 2.0 mm from the epidermis (Fig. 1B). The time constant (TC) and the high cut filter of the amplifier were set at 0.3 seconds and 3 kHz, respectively, and using this amplifier, the action potentials of the pons and medulla oblongata were induced. Moreover, a monitor (Nihon Kohden Co., BSM8302, Tokyo, Japan) was used to monitor and simultaneously record electrocardiograms (ECG).

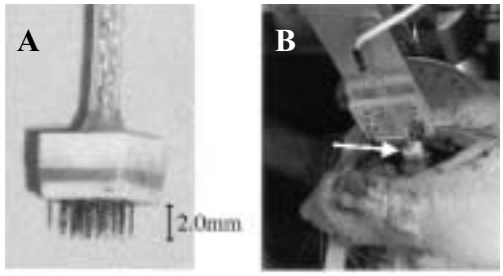


Figure1 : Methods of induction of the action potentials of the medulla oblongata.
(A): A tungsten multimicroelectrode made by accumulating microelectrodes with an apical diameter of 1 μm and an impedance of 3-5 MΩ, and with which it was possible to simultaneously derive 28 potentials, inserted in the sciatic nerve.
(B): A tungsten multimicroelectrode which can derive 28 potentials similarly put into the medulla oblongata to a depth of 2.0mm. as shown by arrow.

From 5 to 6 hours before awakening from anesthesia, analog/digital (A/D) conversion at a sampling frequency of 500 Hz was performed on the data of the action potentials of MSA, the data of the action potentials of the ventral side of the pons and medulla oblongata and the ECG, using a 12 bit A/D conversion board (Keithley Instruments, Inc., KPCM-CIA-16AI-C, Tokyo, Japan). The data of each potential were downloaded into a personal computer (Sony Co., PCG-F37, Tokyo, Japan), and were recorded on the hard disc after digital processing. After full-wave commutation was performed offline, time reset integration was performed every 1 millisecond on the action potentials of MSA, and the action potentials at the resting state were recorded. Moreover, the action potentials of the pons and medulla oblongata were integrated every 1 millisecond, and using spline interpolation, topographic mapping (TM) was performed on the positions of the electrodes in order to record the diachronic changes in the distribution of the action potentials of the ventral side of the pons and medulla oblongata at rest (Fig. 2).

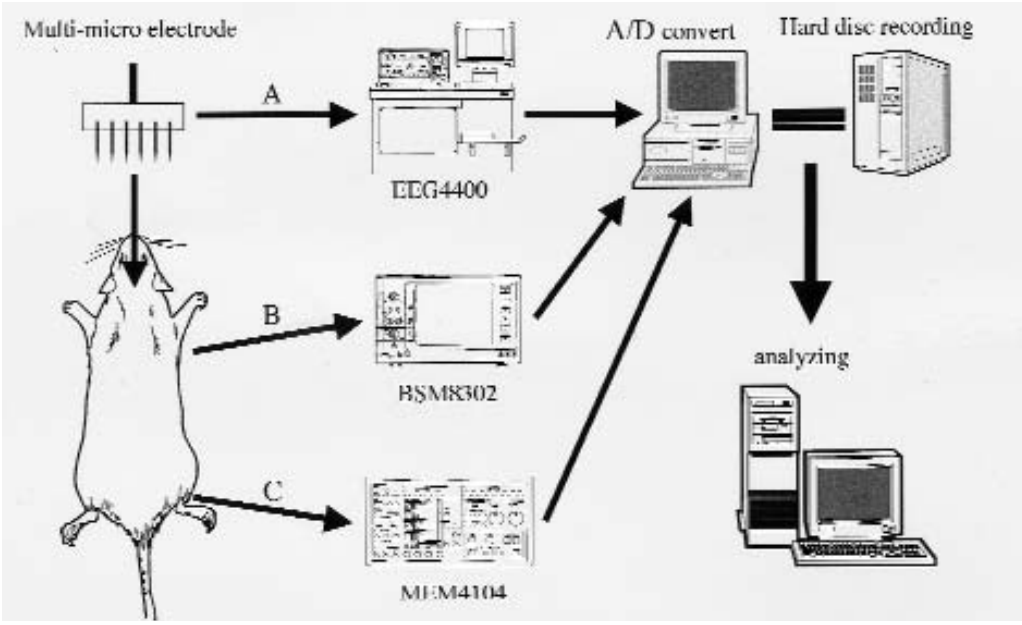


Figure 2 : Recording methods of action potentials.
(A): Action potentials of the medulla oblongata were recorded from a tungsten multimicroelectrode put into the medulla oblongata using an amplifier set at TC = 0.3 and high-frequency cut-off at 3kHz. (B): The ECGs were recorded using an amplifier set at TC = 1.5 and high-frequency cut-off at 60 Hz. (C): The MSA was recorded from the right median nerve using an electromyograph with bandwidth filters set at 500Hz and 5 kHz. Potentials were recorded with a hard disc after A/D conversion at 500 Hz and topographic mapping. ECG = electrocardiogram, MSA = muscle sympathetic nerve activity, EEG4400 = an electroencephalograph (Nihon Kohden Co., EEG4400, Tokyo, Japan), BSM8302 = a monitor (Nihon Kohden Co., BSM8302, Tokyo, Japan), MEM4104 = an electromyograph (Nihon Kohden Co., MEM4104, Tokyo, Japan).

***Results***

1) Action potentials in the pons and medulla oblongata MSA, ECG and action potentials at rest, which were recorded on the ventral side of the pons and medulla oblongata, are shown in Fig. 3A. The derived action potentials, which are designated as 1 to 28, indicate each neural activity on the ventral side of the pons and medulla oblongata. In MSA, regular spontaneous action potentials followed by R-waves in the ECG were detected.

2) Relationship between MSA and each neural activity on the ventral side of the pons and medulla oblongata The diachronic changes in the TM of the action potentials at the resting state, which were recorded on the ventral side of the pons and medulla oblongata during one heartbeat, are shown in Fig. 4. One heartbeat is designated as the distance between 466.0 milliseconds (continuous line a) and 654.0 milliseconds (continuous line b) in Fig. 3A. By integrating TM, the distribution of the action potentials, which diachronically changed during one heartbeat, could be observed. Especially at the time point of 502,0 milliseconds (continuous line c), clear and regular spontaneous action potentials were observed on the ventral side of the pons and medulla oblongata, (Fig. 3B). Action potentials

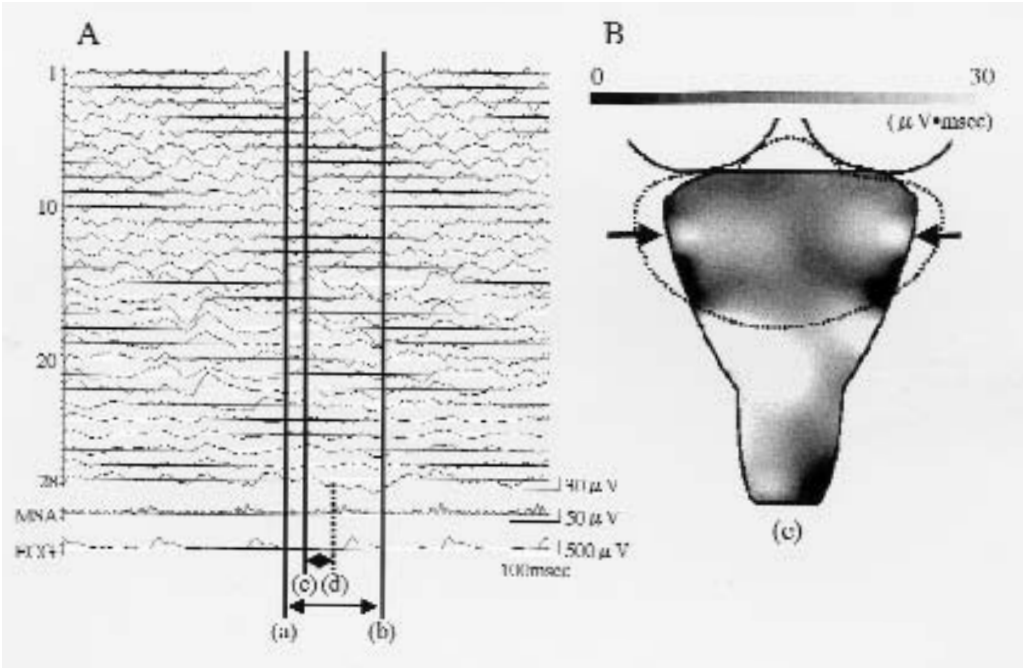


Figure 3 : Potentials and topographic mapping of medulla oblongata.
(A): Potentials showing the waveforms of medulla oblongata, integrated MSA, integrated ECG. From line(a) to line(b) is the duration of one heartbeat. The line(c) is drawn just as action potentials were observed in the ventrolateral medulla oblongata. Line(d) is drawn just where the action potentials of MSA occurred. (B): Topographic mapping of medulla oblongata showed potential on line(c). The spontaneous regular action potentials which synchronize with MSA were observed in the RVLM (shown by arrow. MSA = muscle sympathetic nerve activity, ECG = electrocardiogram, RVLM = rostral ventrolateral medulla oblongata.

were generated in MSA 62.0 milliseconds after generation of the action potentials in the rostral ventrolateral medulla oblongata (RVLM) (dashed line (d)), and this cycle was regularly maintained. The mean interval between generation of clear action potentials on the ventral side of the pons and medulla oblongata and generation of action potentials in MSA was 61.0±1.0 milliseconds.

***Discussion***

It is commonly known that the RVLM plays an important role in blood pressure and its cyclical regulation *(Allen et al., 1988; Maeda et al., 1991).* It is assumed that the neurons in the RVLM can be broadly classified into two types: those which control vasomotion *(Hancock, 1982)* in the skin and muscles and those which control non-vasomotor *(Janig and McLachlan, 1986)* functions of various organs.

By retrograde and antegrade labeling using tracer injections, it has been demonstrated that the efferent fibers originating from the nuclei in the RVLM terminate at the nuclei of the preganglionic sympathetic fibers in the intermediolateral (IML) cell column in the thoracic and lumbar spinal cord *(Amendt et al., 1979).* Destruction and inhibition of cells on

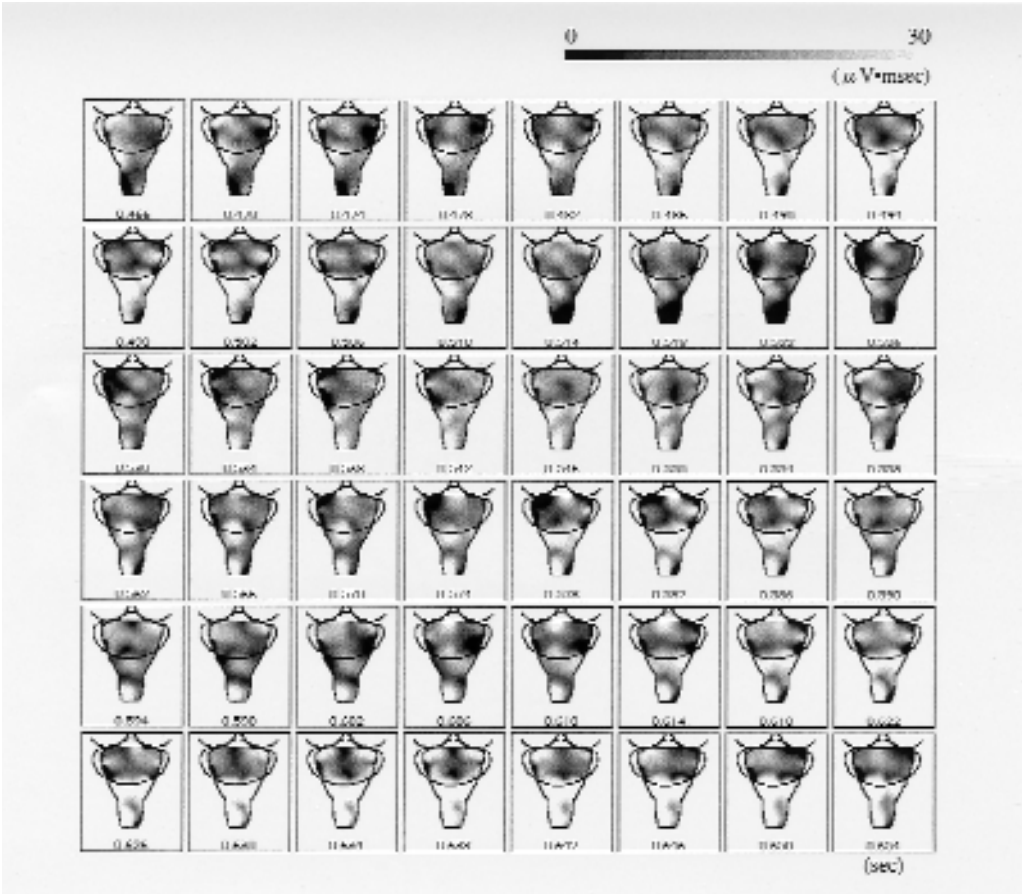


Figure 4 : The change of topographic mapping of the ventral pons and medulla oblongata.
Topographic mapping shown every 4 msec for one beat. The change of action potentials at a depth of 2.0mm in the pons and medulla oblongata were observed using topographic mapping. Action potentials of the RVLM and pyramid were recognized in the pons and medulla oblongata. Especially, the spontaneous regular action potentials were observed in the RVLM. RVLM = rostral ventrolateral medulla oblongata.

both sides of the RVLM decrease the blood pressure, as observed when the spinal cord is severed *(Feldberg and Guertzenstein, 1976; Guertzenstein and Silver, 1974)*. On the other hand, stimulating the cells in the RVLM by topically injecting excitatory amino-acids increases the activities of the vasomotor nerve fibers in the skin and skeletal muscles *(Dampney and McAllen, 1988)*. Consequently, inhibition and stimulation of the cells in the RVLM have immediate and substantial effects on the sympathetic nerve activities, and we therefore suggest that the cells, which control sympathetic functions, exist in the RVLM.

On the other hand, MSA has spontaneous action potentials which synchronize with the heartbeats *(Aramaki et al., 2001),* and play a role in the control of blood volume in the skeletal muscles and in the regulation of blood pressure. In this study, by ana-

lyzing the TM in the pons and medulla oblongata, clear and regular spontaneous action potentials which synchronized with MSA could be observed in the RVLM. Since these action potentials were generated prior to MSA, the regular spontaneous action potentials, which were captured in the RVLM, may have a close relationship with the peripheral vasomotor functions.

It has been suggested that without synaptic inputs, some nuclei in the RVLM can act like a pacemaker and show regular spontaneous activities *(Sun et al., 1988)*.

In this study, we have electrophysiologically demonstrated the existence of premotor nuclei, which generate the peripheral sympathetic vasomotor preganglionic fiber activities at the resting state.

***Acknowlegements***

This work was supported by Grant-in-Aid for Scientific Research (12832042), Japan.

***References***

*Allen AM, MJ McKinley, BJ Oldfield, RA Dampney & FA Mendelsohn:* Angiotensin II receptor binding and the baroreflex pathway.Clin Exp Hypertens. Part A Theory Pract.10 Suppl. 1988, *1*, 63-78.

*Amendt K, J Czachurski, K Dembowsky & H Seller:* Bulbospinal projections to the intermediolateral cell column: a neuroanatomical study. J. Auton. Nerv. Syst. 1979, *1*(1), 103-107.

*Aramaki S, Y Kira, F Nakanishi & Y Hirasawa:* The experimental study on peripheral autonomic nerve potential of the spinal cord injury model by microneurogram method. 1st ISPRM Congress. 2001, *1*, 579-587.

*Burke D, KE Hagbarth & BG Wallin:* Reflex mechanism in Parkinsonian rigidity. Scand. J. Rehabil. Med. 1977, *9*, 15-23.

*Dampney RAL & RM McAllen:* Differential control of sympathetic fibres supplying hindlimb skin and muscle by subretrofacial neurones in the cat. J. Physiol. 1988, *395*, 41-56.

*Feldberg W & PG Guertzenstein:* Vasodepressor effects obtained by drugs acting on the ventral surface of the brain stem. J. Physiol. 1976, *258*(2), 337-355.

*Guertzenstein PG & A Silver:* Fall in blood pressure produced from discrete regions of the ventral surface of the medulla by glycine and lesions. J. Physiol. 1974, *242*(2), 489-503.

*Hagbarth KE, RG Hallin, A Hongell, HE Torebjörk & BG Wallin:* General characteristics of sympathetic activity in human skin nerve. Acta. Physiol. Scand. 1972, *84*, 164-176.

*Hagbarth KE, G Wallin, L Lofstedt & SM Aquilonius:* Muscle spindle activity in alternating tremor of Parkinsonism and clonus. J. Neurol. Neurosurg. Psychiat. 1975, *38*, 636-641.

*Hancock MB:* Separate populations of lumbar preganglionic neurons identified with the retrograde transport of horseradish peroxidase (HRP) and 4,6-diamidino-2-phenylindole (DAPI). J. Auton. Nerv. Syst. 1982, *5*(2), 135-143.

*Janig W:* Organization of the lumbar sympathetic outflow to skeletal muscle and skin of the cat hindlimb and tail. Rev. Physiol. Biochem. Pharmacol. 1985, *102*, 119-213.

*Janig W & EM McLachlan:* I dentification of distinct topographical distributions of lumbar sympathetic and sensory neurons projecting to end organs with different functions in the cat. J. Comp. Neurol. 1986, *246*(1), 104-112.

*Krukoff TL, J Ciriello & FR Calaresu:* Segmental distribution of peptide-like immunoreactivity in cell bodies of the thoracolumbar sympathetic nuclei of the cat. J. Comp. Neurol. 1985, *240*(1), 90-102.

*Maeda M, AJ Krieger, M Nakai & HN Sapru:* Chemical stimulation of the rostral ventrolateral medullary pressor area decreases cerebral blood flow in anesthetized rats. Brain Res. 1991, *563*, 261-269.

*Strack AM, WB Sawyer, LM Marubio & AD Loewy:* Spinal origin of sympathetic preganglionic neurons in the rat. Brain Res. 1988, *455*(1), 187-191.

*Sun MK, JT Hackett J & PG Guyenet:* Sympathoexcitatory neurons of rostral ventrolateral medulla exhibit pacemaker properties in the presence of a glutamate-receptor antagonist. Brain Res. 1988, *438*(1-2), 23-40.

*Vallbo ÅB, KE Hagbarth, HE Torebjörk & BG Wallin:* Somatosensory, proprioceptive, and sympathetic activity in human peripheral nerves. Physiol. Rev. 1979, *59*, 919-957.

*Wallin BG & DL Eckberg:* Sympathetic transients caused by abrupt alterations of carotid baroreceptor activity in humans. Am. J. Physiol. 1982, *242*, 185-190.

*Yatomi A, A Iguchi & K Uemura et al:* A rare case of recurrent vasodepressive attacks of 2-hours duration, analysis of the mechanism by muscle sympathetic nerve activity recording. Clin. Cardiol. 1989, *12*, 164-168.