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Neuroanatomical studies on the mechanism of scalp acupuncture therapy using the pseudorabies virus

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국문초록

Pseudorabies virus(PRV)를 이용한 頭針 治療 機轉에 대한 神經解剖學的 硏究

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본 실험은 pseudorabies 바이러스(PRV)의 Bartha strain을 안면신경의 측두지, 하지를 지배하는 신경(좌골신경) 및 상지를 지배하는 신경(요골, 척골, 정중신경)에 주입한 후 4일간의 생존시간이 경과한 후 척수와 뇌를 적출하여 동결절편을 제작한 후 면역조직화학적 염색기법과 X-gal 조직화학 염색법을 시행하여 염색된 신경세포체를 척수와 뇌에 투사된 공통영역을 관찰하고 두침의 영역중 하나인 운동구와 사지와의 관계에 대한 실험적 증거를 제시하고자 시행하였다.
위의 실험에서 얻어진 결과는 아래와 같다.

1. 안면신경의 측두지, 하지를 지배하는 신경(좌골신경) 및 상지를 지배하는 신경(요골, 척골, 정중신경)에서 투사된 공통된 영역은 척수에서 경수의 층판 I-IV, 흉수의 intermediolateral nucleus(IML), dorsal nucleus(D) 및 층판 X, 요수의 층판 IV, V, 천수의 층판 IV, V, IX, X등의 영역에서 관찰되었고, 뇌줄기에서는 caudoventrolateral reticular nucleus(CVL), nucleus solitary tract(Sol), rostroventrolateral nucleus(RVL), area postrema(AP), raphe nuclei(raphe pallidus, raphe obscurus, raphe magnus), inferior olivary nucleus의 등쪽부분(gigantocellular reticular nucleus, Gi), Kölliker-Fuse nucleus(KF), central gray(CG), dorsal raphe nucleus(DR) and A5 영역에 표지된다. 또한 paraventricular hypothalamic nucleus(PRV)와 lateral hypothalamic reticular nucleus(LH)에서도 관찰되고 locus coeruleus(LC)와 subcoeruleus nucleus(SubCA)에서도 관찰된다.

중심단어(key words) : 두침, 운동구, pseudorabies virus, 좌골신경, 정중신경, 요골신경, 척골신경, 중추신경계

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2. 뇌와 척수에서 공통으로 표지된 영역의 생리적 기능은 안면신경의 측두지(두침영역의 운동구를 지배하는 신경)가 사지에 분포하는 많은 근육들의 운동과 근육을 지배하는 혈관의 운동을 조절하는 기능과 관련이 있음을 제시해 준다.

3. 안면신경의 측두지는 대뇌피질의 상지와 하지의 영역에 표지 되었으며, 이는 두침영역에서 운동구가 사지의 마비와 관련된 질병를 치료할 수 있다는 이론을 형태학적으로 입증하는 증거가 될 것으로 사료된다.

이상의 실험결과는 안면신경의 측두지가 사지의 운동과 관련이 있다는 형태학적 증거이며, 이 신경이 지배하는 두침 영역의 운동구는 사지와 관련이 있을 것으로 생각된다.

I. Introduction

Scalp acupuncture is a therapy for treating disease by needling specific stimulation areas of the scalp. Reported in 1972, this method has been popularized on the basis of the integration of acupuncture therapy and modern medicine on the functional location of the cerebral cortex. Nearly all scalp points are representations of the underlying functional areas of the brain. Therefore, the most common use of scalp acupuncture is to treat conditions in which there is brain damage, such as stroke or severe head injuries, although it can be used for a variety of other conditions[1-3].

During the last thirty years, its clinical use has grown and it has started to spread outside China. It is used to treat diseases in over forty countries. Many clinical reports have sited that more than 80 diseases are treatable by this therapy[2-3]. A study by Wan on the mechanism of the action of scalp acupuncture indicates that cholinesterase is inhibited and, at the same time, muscle force of the extremities is increased. Further, micro-circulation is notably enhanced[4]. But, it is still not clear what the scalp acupuncture's mechanism is nor it's interrelationships with the scalp areas and thelower and upper extremities.

In neuroanatomical study, a few reports have documented that meridian-acupoints-viscera are interrelated using the viral transneuronal labeling method. - the stomach is correlated to Acupoint, ST36[5] and the gall bladder is correlated to Acupoint, GB34[6].

Pseudorabies virus (PRV) is a neurotropic swine alpha herpes virus that characteristically invades the nervous system and replicates within synaptically-linked populations of neurons. The invasive characteristics and ability of this family of viruses to replicate in neurons of the central nervous system (CNS) have been exploited to map functionally related populations of neurons in a variety of systems[7].

The scalp is innervated by the branches of trigeminal nerve, C2, C3, facial nerve and accessory nerve[8]. We selected the temporal branch of the facial nerve innervating a line of the motor area. The motor area is one of 13 treatment areas, created by Shunfa Jiao and is used to treat paralysis of the lower and upper limbs[9]. Also, we selected the sciatic nerve, median nerve, ulnar nerve and radial nerve of the limbs to investigate correlation between the motor area, and the lower and upper limbs.

In this report, the distribution of labeled neurons in the brain and spinal cord observed after injecting the Bartha strain of

pseudorabies virus (PRV) into the temporal branch of the facial nerve, the sciatic nerve, median nerve, ulnar nerve and radial nerve to provide experimental evidence that the motor area of the scalp acupuncture areas is correlated with the lower and upper limbs.

II. Materials and Methods

Animals

Twenty adult Sprague-Dawley rats (250gm B.W) were used in this experiment. Sodium pentobarbital(40mg/kg) was injected intraperitoneally prior to all experimental manipulations. The rat was placed in stereotaxic apparatus and a vertical incision was made along the left preauricular region and parotid region for approximately 2cm. The skin was retracted and underlying tissue was scraped away from the dorsolateral ridge of the frontal bone to the anterior border of the parotid gland. The sciatic nerve was exposed by the incision of the lateral thigh skin and muscles. The median, radial and ulnar nerves were exposed by the incision of the medial brachial skin and muscles.

Virus

Pseudorabies virus, Bartha strain (PRV-Ba) was used in this experiment. This strain of PRV-Ba was cultured in porcine kidney fibroblasts (PK15-cell) and stored at -70℃ in 200$\mu\ell$ aliquots. Fresh aliquots of virus were thawed immediately prior to injection.

The primary antibody was prepared by injecting into the rabbits with acetone-inactivated virus. The immunohistichemical reaction was observed with an ABC kit(Vector lab, USA). PRV-Ba-Gal(PRV mutant expressing functional β-galactosidase), which was obtained from Cheju University.

Neural tracer injections and experimental groups

This experiment was used as a transsynaptic tracers, PRV-Ba and PRV-Ba-Gal, to determine the common locations of the CNS neurons that project to the temporal branch of the facial nerve and the sciatic nerve, respectively. Labeled CNS neurons of the temporal branch of the facial nerve were compare to that of the CNS neurons projecting to the median, radius and ulnar nerve using the neural tracers, PRV-Ba-Gal. Under pentobarbital anesthesia, the temporal branch of facial nerve was exposed and three separate 5 $\mu\ell$ doses of PRV-Ba were injected slowly on the temporal branch with a glass micropipette. After PRV-Ba injection, five microliters of PRV-Ba-Gal were slowly injected into the sciatic nerve. To compare the location of CNS neurons projecting to the upper limb(the median, radial and ulnar nerves) and the temporal branch of the facial nerve. 10$\mu\ell$ of PRV-Ba-Gal was injected into the median, radial and ulnar nerves. The PRV-Ba-Gal labeled areas were compare to those of the temporal branch of the facial nerve.

Experimental procedure

After 4 days survival, the rats were reanesthetized and perfused through the heart with 150ml saline followed by 300ml of 4% paraformaldehyde in 0.1M sodium phosphate buffer(pH 7.4). The brains and spinal cords were removed and stored in a fixative for 1 day, then transferred to 30% sucrose in a 0.1M sodium phosphate buffer(0.1M PB). The brain and spinal cord were cut along transverse planes at 30um on a cryomicrotome. A 1-in 6

series of tissue sections was incubated with a 1:10,000 dilution of rabbit antibody to PRV. The following day, after 2 rinses with 0.1M PB, the sections were reacted with 1:100 biotinylated anti rabbit IgG for 2 hrs in an incubator. After 2hr, the sections were again rinsed and reacted with an avidine-biotin complex(ABC) for PRV-Ba labeled neurons, and PRV-Ba-Gal stained by the X-gal enzymatic staining method[10].

The stained sections were mounted on a gelatin coated slide, air dried, and coverslipped for light microscopy.

III. Results

1. Observation on the PRV-Ba injection into the temporal branch of facial nerve and PRV-Ba-Gal injection into the sciatic nerve

The regions of the spinal cord contained retrogradely commonly labeled neurons following PRV-Ba injection into the temporal branch of facial nerve and PRV-Ba-Gal injection into the sciatic nerve: The cervical spinal cord was detected in the dorsomedial region of lamina I, II, III and IV areas. The thoracic spinal cord was detected in the intermediolateral nucleus(IML), dorsal nucleus and lamina X. The lumbar spinal cord was detected in the dorsolateral region of lamina II, IV, VI, and lamina IX and X regions. The sacral spinal cord was detected in lamina IV, V and VI regions(Fig.1, 5, 6)

The regions of the brain contained retrogradely commonly labeled neurons following PRV-Ba injection into the temporal branch of facial nerve and PRV-Ba-Gal injection into the sciatic nerve. The regions of the medulla oblongata contained retrogradely labeled neurons were detected in the caudoventrolateral reticular nucleus(CVL), the nucleus of the solitary tract(Sol), the area postrema(AP) and the rostroventrolateral reticular nucleus(RVL). In addition, dense labeled neurons were found in raphe pallidus nucleus(RPa), the raphe magnus nucleus(RMg), the raphe obscurus(ROb), the gigantocellular reticular nucleus(Gi, GiA, GiV), the rostroventrolateral reticular nucleus(RVL) and the lateral paragigantocellular nucleus(LPGi)(Fig. 2, 7, 8). The regions of pons containing retrogradely labeled neurons were detected in the A5 region(A5), the locus coeruleus(LC), the subcoeruleus nucleus (SubCA) and the Kölliker-Fuse nucleus(KF) (Fig. 3, 10, 11, 12). The regions of the midbrain containing retrogradely labeled neurons were detected in central gray(CG) and dorsal raphe nucleus(DR)(Fig. 4, 13). The regions of diencephalon contained retrogradely labeled neurons were detected in the paraventricular hypothalamic nucleus(PVN) and the lateral hypothalamic nucleus(LH)(Fig. 4, 14, 15). The regions of the cerebral cortex containing retrogradely labeled neurons were detected in the hindlimb area(HL) and the forelimb area(FL) following only PRV-Ba injection into the temporal branch of the facial nerve(Fig. 4, 16).

2. Observation on the PRV-Ba-Gal injection into the median, radial and ulnar nerves

Labeled neurons in the spinal cord, PRV-Ba-Gal was injected into the median, radial and ulnar nerves were found in the cervical, thoracic, lumbar and sacral spinal segments. Labeled areas of the cervical spinal cord segment were found in lamina I, II, III, IV and X(Fig. 1). Labeled areas of the thoracic

spinal cord were found in the intermediolateral nucleus(IML), lamina IV, V and X areas(Fig. 1, 17). Labeled areas of the lumbar spinal cord were found in lamina IV, V, and VII areas(Fig. 1). Labeled areas of the sacral spinal cord were lamina IV, V, VI and IX areas(Fig. 1).

Labeled neurons in the medulla oblongata were found in the A1 noradrenalin cells/C1 adrenalin cells/caudoventrolateral reticular nucleus, the rostroventrolateral reticular nucleus, the nucleus tractus solitarius(Sol), the raphe obscurus nucleus, the raphe pallidus nucleus, the raphe magnus nucleus, the gigantocellular reticular nucleus(GiA) and the lateral paragigantocellular nucleus(Fig. 2, 3, 18, 19). PRV-Ba-Gal labeled neurons in the pons were found in the locus coeruleus(LC) and the A5 cell group(A5)(Fig. 4, 20, 21). PRV-Ba-Gal labeled neurons in the midbrain were found the central gray. PRV-Ba-Gal labeled neurons in the diencephalon were found in the paraventricular hypothalamic nucleus(Fig. 4, 22).

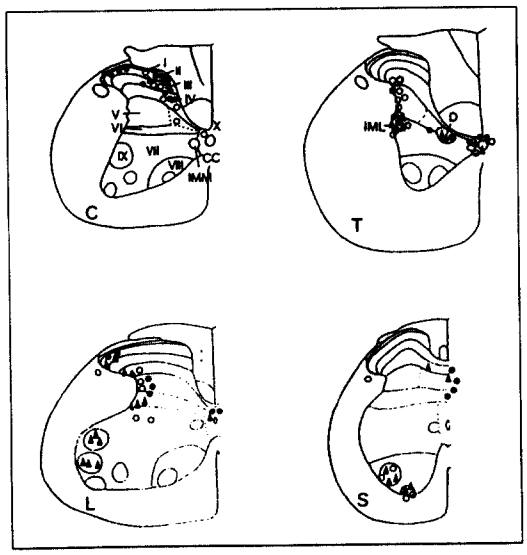

Fig 1. Projection drawings of coronal section taken from cervical(C), thoracic(T), lumbar(L) and sacral(S) spinal cord following PRV-Ba injection into the temporal branch of facial nerve(●), PRV-Ba-Gal injection into the sciatic nerve(▲) and PRV-Ba-Gal injection into the median, radial and ulnar nerves(○). Commonly labeled neurons were observed in lamina I, II, III and IV. ●, ▲, ○, labeled neurons. I, II, III, IV, V, VI, VII, VIII, IX, X, lamina area; CC, central canal; D, dorsal nucleus; IMM, intermediomedial nucleus; IML, intermediolateral nucleus.

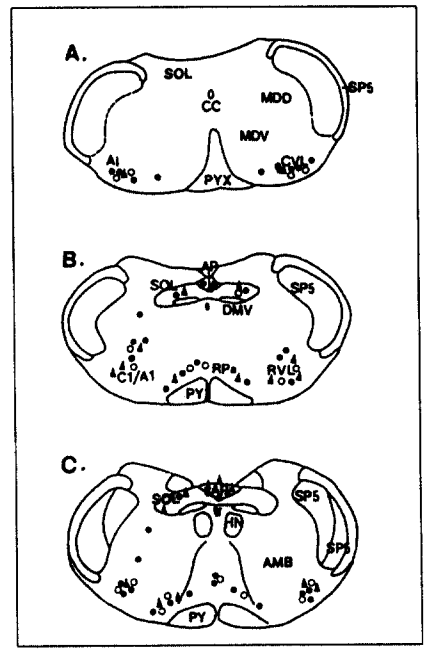

Fig. 2. Projection drawings of coronal section taken from rostral(A) to middle(C) level of the medulla oblongata following PRV-Ba injection into the temporal branch of facial nerve(●) and PRV-Ba-Gal injection into the sciatic nerve(▲) and PRV-Ba-Gal injection into the median, radial and ulnar nerves(○). Commonly labeled neurons were observed in CVL, RVL and SOL. ●, ▲, ○, labeled neurons.

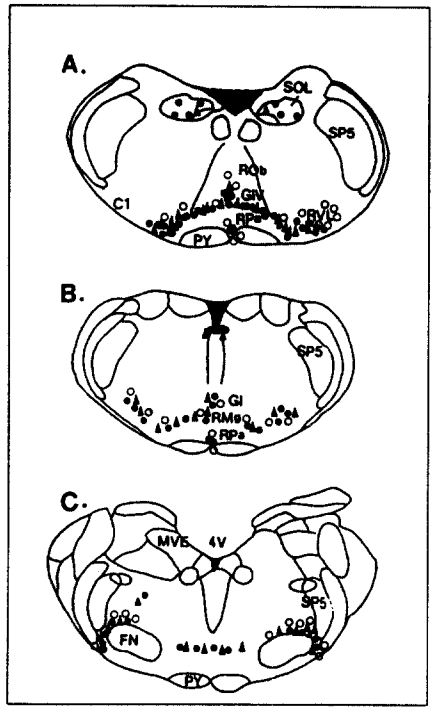

Fig. 3. Projection drawings of coronal section taken from middle(A) to caudal(C) level of the medulla oblongata following PRV-Ba injection into the temporal branch of facial nerve(●) and PRV-Ba-Gal injection into the sciatic nerve(▲) and PRV-Ba-Gal injection into the median, radial and ulnar nerves(○). Commonly labeled neurons were observed in SOL, Gi, RPa, RMg, ROb and A5 region. ●, ▲, ○, labeled neurons.

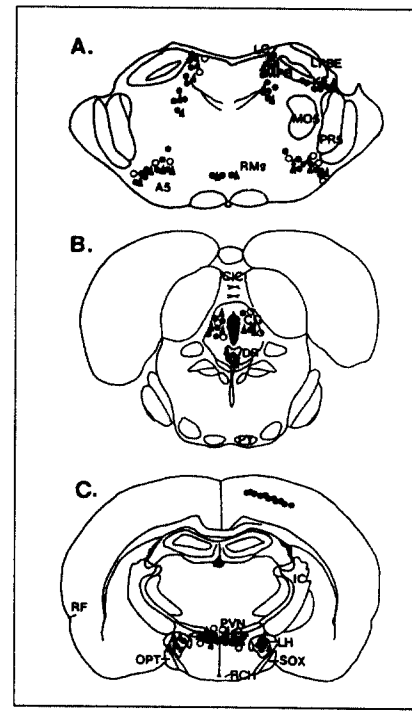

Fig. 4. Projection drawings of coronal section taken from pons(A), midbrain(B) and prosencephalon(C) level of the brain following PRV-Ba injection into the temporal branch of facial nerve(●) and PRV-Ba-Gal injection into the sciatic nerve(▲) and PRV-Ba-Gal injection into the median, radial and ulnar nerves(○). Commonly labeled neurons were observed in LC, CG and PVN. ●, ▲, ○. labeled neurons.

V. Discussion

Scalp acupuncture refers to the therapeutic approach of needling specific stimulation areas of the scalp. It is often applied for cerebral diseases. The needling points are scalp areas corresponding to functional areas of the cerebral cortex, the nomenclature of the areas(lines) are in accordance with the functional area of the cerebral cortex[11].

Huang Xuelong in 1935 introduced the concept that there is a relationship between the scalp and cerebral cortex. During the 1970's, scalp acupuncture developed as a complete acupuncture system. Four major contributors to the development of this system, Shunfa Jiao, Yunpeng Fang, Songyan Tang, and Mingqing Zhu, proposed different diagrams and groupings of scalp acupuncture points[8]. In this report, Jiao's scalp acupuncture system was used.

Jiao's scalp acupuncture is composed of a motor area, a sensory area, a chorea and a tremor control area, a vasomotor area, a foot motor-sensory area, a vertigo and auditory area, a 2nd speech area, a 3rd speech area, a praxis area, an optic area, a balance area, a gastric area, a genital area, a thoracic cavity area, etc[8,12,13]. It is mainly indicated for cerebral disorders, such as hemiplegia, numbness, aphasia, dizziness and vertigo, tinnitus, chorea, etc. It is also applied for headaches, lower back and leg pain, nocturia, trigeminal neuralgia, scapulohumeral periarthritis and other diseases of the nervous system[1,11].

In this report, the motor area of scalp acupuncture areas was selected to investigate the relationship dyskinesia of the limbs and scalp acupuncture after stroke. The motor area is located over the anterior central convolution of the cerebral cortex. The point 0.5 cm posterior to the midpoint of the anterior-posterior line defines the upper limit of the motor area. The lower limit intersects the eyebrow-occiput line at the anterior border of the natural hairline on the temple. The connection line between these two points is the motor area. The upper 1/5 represents the lower limbs and trunk, the middle 2/5 represents the upper limbs, and the lower 2/5 represents the face. This area is used to treat contralateral motor disturbance of the appropriate area[9,11,12]. The motor area of the scalp acupuncture areas is innervated by the trigeminal nerve and the facial nerve[8].

This experiment was used as transsynaptic tracers, PRV-Ba and PRV-Ba-Gal, to determine the common locations of the CNS neurons that project to the temporal branch of the facial nerve and the sciatic nerve, respectively, and labeled CNS neurons of the temporal branch of the facial nerve were compare to that of the CNS neurons projecting to the median, radial and ulnar nerves using the neural tracer, PRV-Ba-Gal.

The present study was undertaken to accomplish two goals: (1) to identify the common locations of CNS neurons projecting to the temporal branch of the facial nerve, nerve innervating the hindlimb(sciatic nerve) and nerves innervating the forelimb(median nerve, ulnar nerve and radial nerve) and (2) to identify the relationship between neurons projecting to the motor area of the scalp acupuncture areas(the temporal branch of the facial nerve) and neurons projecting to nerves innervating the lower and upper extremities.

PRV labeled cells, projecting to the temporal branch of the facial nerve, the nerve innervating hind limb(sciatic nerve) and the nerves innervating upper limb(median nerve, ulnar nerve and radial nerve), were commonly found bilaterally in the cervical segments, particularly densely labeled neurons were found in lamina I, II, III and IV of dorsal horn. In the thoracic segments, commonly labeled neurons were found in the intermediolateral nucleus(IML), the dorsal nucleus(D) or the central autonomic nuclei of lamina VII and dorsal to the central canal(lamina X). In the lumbar segments, commonly labeled neurons were found in the dorsolateral part of the dorsal horn, particularly densely labeled in lamina IV and V. In the sacral segments, labeled neurons were scattered in lamina IV, V, IX and X. In the brainstem, common cell body labeling was confined mainly to the autonomic premotor nuclei including the caudoventrolateral reticular nucleus(CVL), the nucleus solitary tract(Sol), the

rostroventrolateral nucleus(RVL), the area postrema(AP), the raphe nuclei(raphe pallidus, raphe obscurus, magnus), the the area dorsal to the inferior olivary nucleus(gigantocellular reticular nucleus, Gi), the Kölliker-Fuse nucleus(KF), the central gray(CG), the dorsal raphe nucleus(DR) and the A5 cell group. They were also found in the the paraventricular hypothalamic nucleus(PRV) and the lateral hypothalamic reticular nucleus(LH). Cell body labeling was found in the other areas which are thought to project directly to motorneurons; these include the locus coeruleus(LC) and the subcoeruleus nucleus(SubCA).

Insight to the function of common locations in the above data may be derived from previous physiological studies.

Interneurons most extensively studied in above experiments have been those involved in reflex motor function. These include Renshaw cells which are localized mainly in the ventral part of lamina VII[13,14]. These interneurons may serve to coordinate the motor activity of multiple muscles that are operative at different joints of the hindlimb which are important in walking or standing[15].

Rotto-Percelay et. al.[16] provides direct anatomical evidence that the sympathetic control of blood vessels in a single hindlimb muscle originates from five spinal segments(T11-L2) with T13 being the major source, and the IML proper, as opposed to the more medial spinal cord sites that contain sympathetic preganglionic neurons like the central autonomic nucleus near the central canal, is the primary site where these cells are found. Jänig and McLachlin[17] who observed that after HRP injections into the lumbar sympathetic chain, retrogradely labeled neurons were concentrated in the lateral border of the spinal gray matter(i.e. IML) as opposed to the medial zone of lamina VII.

Since sympathetic neurons of the lumbar sympathetic chain innervate the blood vessels of hindlimb muscle, they deduced that the site of origin of the sympathetic preganglionic neurons was IML.

Spinal motorneurons(lamina IX) receive direct inputs from the locus coeruleus, nucleus subcoeruleus, raphe nuclei and reticular formation.

In this study, viral labeled cells were identified in all of these sites as well as other nuclei including the rostral ventrolateral medulla, A5 region, central gray, dorsal raphe nucleus, paraventricular hypothalamic nucleus and lateral hypothalamic nucleus. Since skeletal muscles of upper and lower extrimities are innervated by both the somatic and sympathetic pathways, both could serve as conduits for the entry of PRV into the spinal cord. As expected, the corresponding brainstem nuclei of both motor systems were transneuronally labeled. However, our data do not permit us to discriminate between which groups of CNS neurons control somatomotor functions and which control sympathetic function. Many of the lower brainstem nuclei provide dual descending projections to both sympathetic and somatic motor outflows. For example, it has been demonstrated with anterograde transport methods that raphe nuclei, adjacent medial reticular formation, and ventral medulla provide direct inputs to the IML as well as to the ventral horn[18,19].

It is not known whether single medullary neurons provide dual inputs to the somatic and sympathetic preganglionic motorneurons or if these are separate descending parallel

pathways arising from these brainstem nuclei. In either event, it is possible that one or more of these brainstem sites function to synchronize somatomotor and sympathetic functions such as would be seen in the sleep-waking cycle or under emergency conditions associated with fight or flight. However, some of the other labeled cell groups project only to IML- such as A5 cell group[19] or predominantly to the IML- such as paraventricular hypothalamic nucleus[20].

These cell groups were consistently labeled in our experiments and almost surely, the A5 cell group is involved in sympathetic regulation of blood flow in skeletal muscle[21]. Similarly, the rostral ventrolateral medulla was consistently labeled, and there is excellent evidence to suggest that this vasomotor center regulates blood flow in hindlimb muscle[22].

The periaqueductal gray matter(central gray, PAG) has come under investigation for its role in autonomic control only recently. Prominent fiber system running through the PAG were connect to forebrain and brainstem autonomic control nuclei. Electrical or chemical stimulation of the PAG can produce autonomic, somatomotor, or antinociceptive effets[23].

The locus coeruleus was concerned in playing a major role in preparing the cerebral cortex for efficient processing of sensory stimuli during arousal. However, legions of the locus coeruleus fail to produce major changes in the level of consciousness or wake-sleep cycle in cats[24].

These physiological functions of PRV labeled neurons in the brain and spinal cord provide experimental evidence that the temporal branch of the facial nerve, innervating the motor area of the scalp acupuncture areas, is correlated with regulations of motor activity of multiple muscle and blood flow in the lower and upper limbs.

PRV labeled cells in the cerebral cortex projecting to the temporal branch of facial nerve were detected in the hindlimb area(HL) and the forelimb area(FL). It demonstrates the existence of previously unrecognized polysynaptic pathways in the rat CNS, which extend from the brain cortex to the temporal branch of the facial nerve. Also, it provides experimental evidence that temporal branch of facial nerve is correlated with hindlimb area(HL) and forelimb area(FL) of the cerebral cortex and, motor area of scalp acupuncture areas, innervated by the temporal branch of the facial nerve, used to treat paralysis of the lower and upper limbs.

V. Conclusions

The common locations of the spinal cord and brain projecting to the temporal branch of the facial, sciatic, median, radial and ulnar nerves were studied with injection of pseudorabies virus(PRV-Ba and PRV-Ba-Gal) into these nerves to identify the common locations of CNS neurons and to provide experimental evidence that the motor area of the scalp acupuncture areas is correlated with lower and upper limbs.

The obtained results were as follows :

1. PRV labeled common cells were found in the cervical segments, particulary densely labeled neurons were founded in lamina I, II, III and IV of the dorsal horn. In the thoracic segments, commonly labeled neurons were found in the intermediolateral nucleus(IML), the

dorsal nucleus(D) or the central autonomic nuclei of lamina VII and dorsal to the central canal(lamina X). In the lumbar segments, commonly labeled neurons were found in the dorsolateral part of the dorsal horn, particularly densely labeled in lamina IV and V. In the sacral segments, labeled neurons were scattered in lamina IV, V, IX and X. In the brainstem, common cell body labeling was confined mainly to the autonomic premotor nuclei including the the caudoventrolateral reticular nucleus(CVL), the nucleus solitary tract(Sol), the rostroventrolateral nucleus(RVL), the area postrema(AP), the raphe nuclei(raphe pallidus, raphe obscurus, magnus), the the area dorsal to the inferior olivary nucleus(gigantocellular reticular nucleus, Gi), the Kölliker-Fuse nucleus(KF), the central gray(CG), the dorsal raphe nucleus(DR) and the A5 cell group. They were also found in the paraventricular hypothalamic nucleus(PRV) and the lateral hypothalamic reticular nucleus(LH). Cell body labeling was found in the other areas which are thought to project directly to motorneurons; these include the locus coeruleus(LC) and the subcoeruleus nucleus(SubCA).

2. These physiological functions of PRV labeled common neurons in the brain and spinal cord provide experimental evidence that the temporal branch of the facial nerve, innervating the motor area of the scalp acupuncture areas, is correlated with regulations of motor activity of multiple muscle and blood flow in the lower and upper limbs.

3. The temporal branch of the facial nerve is projected to the hindlimb area(HL) and the forelimb area(FL) of the cerebral cortex. And, it provides the experimental evidence that the motor area of the scalp acupuncture areas, innervated by the temporal branch of the facial nerve, can be used to treat paralysis of the lower and upper limbs.

These findings provide the first neuroanatomical evidence that the temporal branch of the facial nerve is correlated with motor activity of the extremities. Also, the motor area of the scalp acupuncture areas, innervated by the temporal branch of the facial nerve, is correlated with the area projecting to the extremities.

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Legends for Figures

Fig. 5. Distribution of PRV-Ba(dark brown reaction) and PRV-Ba-Gal(blue reaction, arrow) labeled neurons in cervical spinal cord following PRV-Ba injection into temporal branch of facial nerve and PRV-Ba-Gal injection into the sciatic nerve. Commonly labeled neurons were detected in lamina I and II of dorsal horn; arrow, blue reaction.

Fig. 6. Distribution of PRV-Ba(dark brown reaction) and PRV-Ba-Gal(blue reaction, arrow) labeled neurons in thoracic spinal cord following PRV-Ba injection into temporal branch of facial nerve and PRV-Ba-Gal injection into the sciatic nerve. Commonly labeled neurons were detected in lamina X and intermediolateral nucleus(IML). CC, central canal; arrow, blue reaction.

Fig. 7. Distribution of PRV-Ba(dark brown reaction) and PRV-Ba-Gal(blue reaction, arrow) labeled neurons in medulla oblongata following PRV-Ba injection into temporal branch of facial nerve and PRV-Ba-Gal injection into the sciatic nerve. Commonly labeled neurons were detected in ROb(raphe obscurus nucleus) and GiV(gigantocellular reticular nucleus, ventral); arrow, blue reaction.

Fig. 8. Distribution of PRV-Ba(dark brown reaction) and PRV-Ba-Gal(blue reaction, arrow) labeled neurons in medulla oblongata following PRV-Ba injection into temporal branch of facial nerve and PRV-Ba-Gal injection into the sciatic nerve. Commonly labeled neurons were detected in Sol(nucleus solitary tract); arrow, blue reaction.

Fig. 9. Distribution of PRV-Ba(dark brown reaction) and PRV-Ba-Gal(blue reaction, arrow) labeled neurons in medulla oblongata following PRV-Ba injection into temporal branch of facial nerve and PRV-Ba-Gal injection into the sciatic nerve. Commonly labeled neurons were detected in RMg(raphe magnus nucleus), RPa(raphe pallidus nucleus) and GiA(gigantocellular reticular nucleus, alpha); arrow, blue reaction.

Fig. 10. Distribution of PRV-Ba(dark brown reaction) and PRV-Ba-Gal(blue reaction, arrow) labeled neurons in pons following PRV-Ba injection into temporal branch of facial nerve and PRV-Ba-Gal injection into the sciatic nerve. Labeled neurons were detected in LC(locus coeruleus). 4V, fourth ventricle; arrow, blue reaction.

Fig. 11. Distribution of PRV-Ba(dark brown reaction) and PRV-Ba-Gal(blue reaction, arrow) labeled neurons in pons following PRV-Ba injection into temporal branch of facial nerve and PRV-Ba-Gal injection into the sciatic nerve. Commonly labeled neurons were detected in A5(A5 group). 7n, seventh cranial nerve; arrow, blue reaction.

Fig. 12. Distribution of PRV-Ba(dark brown reaction) and PRV-Ba-Gal(blue reaction, arrow) labeled neurons in pons following PRV-Ba injection into temporal branch of facial nerve and PRV-Ba-Gal injection into the

sciatic nerve. Commonly labeled neurons were detected in LC(locus coeruleus) and SubCA(subcoeruleus, alpha). scp, superior cerebellar peduncle; arrow, blue reaction.

Fig. 13. Distribution of PRV-Ba(dark brown reaction) and PRV-Ba-Gal(blue reaction, arrow) labeled neurons in midbrain following PRV-Ba injection into temporal branch of facial nerve and PRV-Ba-Gal injection into the sciatic nerve. Commonly labeled neurons were detected in CG(central gray) and DR(dorsal raphe nucleus); arrow, blue reaction.

Fig. 14. Distribution of PRV-Ba(dark brown reaction) and PRV-Ba-Gal(blue reaction, arrow) labeled neurons in diencephalon following PRV-Ba injection into temporal branch of facial nerve and PRV-Ba-Gal injection into the sciatic nerve. Commonly labeled neurons were detected in PVN(paraventricular hypothalamic nucleus). 3V, third ventricle; arrow, blue reaction.

Fig. 15. Distribution of PRV-Ba(dark brown reaction) and PRV-Ba-Gal(blue reaction, arrow) labeled neurons in diencephalon following PRV-Ba injection into temporal branch of facial nerve and PRV-Ba-Gal injection into the sciatic nerve. Commonly labeled neurons were detected in LH(lateral hypothalamic nucleus). sox, supraoptic decussation; arrow, blue reaction.

Fig. 16. Distribution of PRV-Ba(dark brown reaction) labeled neurons in cerebral cortex following PRV-Ba injection into temporal branch of facial nerve. PRV-Ba labeled neurons were detected in HL(hindlimb area) and FL(forelimb area).

Fig. 17. Distribution of PRV-Ba-Gal(blue reaction) labeled neurons in thoracic spinal cord following PRV-Ba-Gal injection into median, radial and ulnar nerves. Labeled neurons were detected in intermediolateral nucleus(IML); CC, central canal.

Fig. 18 .Distribution of PRV-Ba-Gal(blue reaction) labeled neurons in medulla oblongata following PRV-Ba-Gal injection into the median, radial and ulnar nerves. Labeled neurons were detected in Sol(nucleus solitary tract).

Fig. 19. Distribution of PRV-Ba-Gal(blue reaction) labeled neurons in medulla oblongata following PRV-Ba-Gal injection into the median, radial and ulnar nerve. Labeled neurons were detected in RMg(raphe magnus nucleus), RPa(raphe pallidus nucleus), rostroventrolateral reticular nucleus(RVL) and GiA(gigantocellular reticular nucleus, alpha).

Fig. 20. Distribution of PRV-Ba-Gal(blue reaction) labeled neurons in pons following PRV-Ba-Gal injection into the median, radial and ulnar nerves. Labeled neurons were detected in LC(locus coeruleus).

Fig. 21. Distribution of PRV-Ba-Gal(blue reaction) labeled neurons in pons following PRV-Ba-Gal injection into the median, radial and ulnar nerves. Labeled neurons were detected in A5(A5 group); 7n, seventh cranial nerve.

Fig. 22. Distribution of PRV-Ba-Gal(blue reaction) labeled neurons in diencephalon following PRV-Ba-Gal injection into the median, radial and ulnar nerves. Labeled neurons were detected in PVN(paraventricular hypothalamic nucleus); 3V, third ventricle.