

# SOMATOTOPIC ORGANIZATION OF THE FACIAL NUCLEUS IN THE MONKEY AND THE RABBIT

Ph. D. Thesis

by

# TAKAHIRO SATODA, D. D. S.

Hiroshima University School of Dentistry

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I. Representation of the main branches of the facial nerve within the facial nucleus of the Japanese monkey (<u>Macaca</u> <u>fuscata</u>)

Takahiro Satoda, Osamu Takahashi, Takashi Tashiro, Ryotaro Matsushima, Masanori Uemura-Sumi, and Noboru Mizuno

Neuroscience Letters 78: 283-287, 1987.

II. Somatotopic organization of facial nucleus of rabbit, with particular reference to intranuclear representation of perioral branches of the facial nerve

Takahiro Satoda, Osamu Takahashi, Takashi Tashiro, Ryotaro Matsushima, Masanori Uemura-Sumi, and Noboru Mizuno

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Anatomischer Anzeiger 165: 83-90, 1988.

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#### I. INTRODUCTION

Somatotopic organization of the facial nucleus has been studied in a variety of mammals by the morphological and electrophysiological techniques (Yagita, 1910; Szentágothai, 1948; Kitai et al., 1972; Dom et al., 1973; Dom and Zielinsky, 1977; Kume et al., 1978; Shohara and Sakai, 1983; Uemura-Sumi et al., 1986. for further reviews, cf. Papez, 1927; Vraa-Jensen, 1942; Courville, 1966; Martin and Lodge, 1977; Dom, 1982; Hinrichsen and Watson, 1984, Baisden et al., 1987). The previous morphological studies usually used transection of individual facial nerve branches and retrograde degeneration techniques for identification of facial motoneurons which send their axons to the facial nerve branches (for reviews, cf. Yagita, 1910; Papez, 1927; Vraa-Jensen, 1942; Courville, 1966). More recently, the retrograde HRP (horseradish peroxidase) method has been developed (for review, cf. Mesulam, 1982). Since this method was shown to enable an easier and clearer identification of subgroups of motoneurons to be made (Mizuno et al., 1975), a group of studies have been carried out on the myotopic organization of the facial nucleus after injection of HRP into the facial muscles in the opossum (Dom and Zielinsky, 1977; Provis, 1977), bat (Friauf and Herbert, 1985), mouse (Ashwell, 1982; Komiyama et al., 1984), rat (Watson et al., 1982; Hinrichsen and Watson, 1984; Friauf and

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Herbert, 1985), kitten (Radpour, 1977), cat (Kume et al., 1978; Parnes et al., 1982), and rhesus and cynomolgus monkeys (Parnes et al., 1982). Correlation of the subdivisions of the facial nucleus with the peripheral branches of the facial nerve has also been examined after applying HRP to the facial nerve branches in 1982), the opossum (Dom, rat (Semba, 1984), guinea pig (Uemura-Sumi et al., 1986), and rabbit (Baisden et al., 1987). The results of the previous studies indicate that there more or less exist species differences in the somatotopic organization of the facial nucleus.

The somatotopic organization of the facial nucleus of the macaque monkeys has not been systematically studied by the HRP method. The present study, therefore, was attempted to examine the correlation of the main peripheral branches of the facial nerve with cytoarchitectonic divisions of the facial nucleus in the Japanese monkey (<u>Macaca fuscata</u>) to shed more light on the general organization of the facial nucleus in mammalian species. For the purpose of comparison, the study was also performed in the rabbit as a representative of the most common laboratory animals, although a study in the rabbit has already been reported (Baisden et al., 1987).

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# **II. MATERIALS AND METHODS**

The present reports are based on the results which were obtained from 18 Japanese monkeys (<u>Macaca fuscata</u>) weighing 3 – 12 kg, and 35 adult rabbits weighing 1.8 – 3.0 kg. The experiments were performed according to the NIH Guide for the Care and Use of Laboratory Animals (National Institute of Health Publications No. 85-83, Revised 1985). Horseradish peroxidase (HRP) was purchased from Toyobo, Japan (HRP, Grade-1-C: RZ= 3.3 or 3.4).

## 1. Application of HRP

### 1) monkey

The monkeys were anesthetized with an intraperitoneal injection of sodium pentobarbital (30 - 35 mg/kg body weight). Supplementary doses were given as necessary to maintain a deep level of anesthesia during long surgical procedures.

In 12 monkeys, the main branches of the facial nerve supplying the superficial facial muscles were exposed unilaterally with an aid of an operation microscope. They were cut, and both proximal and distal cut-ends were ligated (Fig. 1a). Subsequently, at the sites close to the proximal cut-end,

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multiple injections of the total of 0.1 - 0.5  $\mu$ l of 30% HRP dissolved in 0.9% saline were made manually by pressure through a glass micropipette (tip diameter: 40 - 60  $\mu$ m) coupled to a 10- $\mu$ 1 Hamilton microsyringe; the main branches other than the target nerve branch had been cut and ligated with thin thread before the HRP injection into the target nerve branch. The proximal cut-end of the target nerve branch injected with HRP was further inserted into a piece of polyethylene tube which was filled with 30% HRP dissolved in sterile 0.9% saline. The open end of the tube was then sealed with vaseline. Subsequently, the tube was fixed to the neighboring region with a quick set adhesive (Alon Alfa; alpha-cyanoacrylate).

In 3 monkeys, the HRP solution was applied to the trunk of the facial nerve at the site close to the stylomastoid foramen.

In additional 3 monkeys, 5  $\mu$ l of 30% HRP dissolved in 0.9% saline was injected into the orbicularis oculi muscle after severing and ligating nerve branches supplying the superficial facial muscles in the anterior auricular and the superior labial regions.

### 2) Rabbit

The rabbits were anesthetized with an intraperitoneal injection of urethane (1 g/kg body weight), followed with an intravenous injection of sodium pentobarbital (25 - 30 mg/kg body

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weight). Supplementary doses of sodium pentobarbital were given when necessary throughout the duration of the experiments.

In 17 rabbits, the main peripheral branches of the facial nerve were exposed unilaterally under an operation microscope (Fig. 2a). They were cut, and both proximal and distal cut-ends were ligated. Subsequently, at the sites close to the proximal cut-end, a single main branch of the facial nerve was injected with 0.1 - 0.5  $\mu$ l of 30% HRP dissolved in sterile 0.9% saline manually by pressure through a glass micropipette (tip diameter: 40 - 60  $\mu$ m) coupled to a 10- $\mu$ l Hamilton microsyringe. The proximal cut-end of the target nerve branch injected with HRP was further inserted into a piece of polyethylene tube filled with 30% HRP dissolved in sterile 0.9% saline. The open end of the target nerve branch injected with HRP was further inserted into a piece of polyethylene tube filled with the main the sealed with vaseline, and the tube was fixed to the neighboring region by Alon Alfa.

In 2 rabbits, the application of the HRP solution was performed to the trunk of the facial nerve at the sites close to the stylomastoid foramen (Fig. 2a).

In each of 12 rabbits, the application of the HRP solution was done to one of the 4 perioral branchlets arising from the superior labial branch, and/or the inferior labial branch (Fig. 3a).

In 4 rabbits, the HRP solution was injected unilaterally into the superficial facial muscles in the periorbital region

after cutting and ligating the superior labial and the inferior labial branches of the facial nerve.

## 2. Perfusion

After the application of HRP to the facial nerve or muscles, the animals were allowed to survive for 36-48 hours, and then re-anesthetized deeply with an overdose of the anesthesia, and were perfused through the ascending aorta with 9% formalin in 0.1 M phosphate buffer (pH 7.3) (1 1/kg body weight), followed by the same buffer containing 10% sucrose (0.5 1/kg body weight).

### 3. Histological procedures

After the transcardial perfusion, the brainstems were removed and placed into 30% sucrose in 0.1 M phosphate buffer (pH 7.3) at 4°C. When the brainstems were saturated with the solution, they were cut into transverse serial sections of 60  $\mu m$ thickness on freezing microtome. For the a histochemical demonstration of HRP, the sections were treated with

tetramethylbenzidine (TMB) according to Mesulam (1978). Then the sections were mounted on glass slides coated with a chrome alum-gelatine (Pappas, 1971), and counterstained with 1% neutral red.

4. Observation of the facial nerve

After the perfusion and removal of the brainstem, the pattern of branching of the facial nerve supplying the superficial facial muscles were examined macroscopically with an aid of an operation microscope.

5. Observation of HRP-labeled neurons

The serial sections were examined microscopically in bright-field and dark-field illumination. The distribution of neuronal cell bodies labeled retrogradely with HRP was charted onto enlarged outline drawings of representative sections that were enlarged by using a projection apparatus.

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#### III. Results

#### 1. Monkey

In all monkeys examined in the present study, 5 main peripheral branches of the facial nerve were identified, although the pattern of branching of the facial nerve was variant from animal to animal (Fig. 1a). The 5 main peripheral branches were the cervical (Ce). the posterior auricular (PA), the auriculo-zygomatico-orbital (AZO), the superior labial (SL), and the inferior labial (IL) branches. The auriculo-zygomatic-orbital (AZO) branch was composed of several nerve branchlets supplying the anterior auricular (AA) and zygomatico-orbital (ZO) regions.

In 3 monkeys, HRP was applied to the trunk of the facial nerve. In these monkeys, the facial nucleus ipsilateral to the HRP application was filled with neuronal cell bodies which were labeled retrogradely with HRP (Fig. 4a). No HRP-labeled neuronal cell bodies were found on the side contralateral to the HRP application. The facial nucleus filled with HRP-labeled motoneurons was divided into 5 divisions; into the ventral (V), medial (M), intermediate (I), dorsal (D), and lateral (L) divisions (Figs. 1b-d and 4a). Facial motoneurons in the ventral division were clustered into 2 subgroups at the level of the

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caudal two thirds of the facial nucleus (Figs. 1c, d and 4b). In 2 monkeys which were applied with HRP to the cervical (Ce) branch of the facial nerve, HRP-labeled neuronal cell bodies were seen ipsilaterally in the ventral (V) division of the facial nucleus ipsilateral to the HRP application. Both of the 2 subgroups of facial motoneurons in the ventral division were labeled with HRP (Figs. 1b-d and 4b).

In 2 monkeys which were applied with HRP to the posterior auricular (PA) branch of the facial nerve, neuronal cell bodies labeled with HRP were observed ipsilaterally in the medial (M) division of the facial nucleus (Figs. 1b-d and 4c).

In 3 monkeys which were applied with HRP to the auriculo-zygomatico-orbital (AZO) branch of the facial nerve, HRP-labeled neuronal cell bodies were seen ipsilaterally in the intermediate (I) and dorsal (D) divisions of the facial nucleus. In one of the 3 monkeys, a few HRP-labeled neurons were distributed in the dorsolateral aspects of the medial (M) division of the facial nucleus (Fig. 1b-d).

In 3 monkeys which were applied with HRP to the superior labial (SL) branch of the facial nerve, HRP-labeled neuronal cell bodies were seen ipsilaterally in the dorsal (D) and lateral (L) divisions of the facial nucleus (Figs. 1b-d and 4d).

In 2 monkeys which were applied with HRP to the inferior labial (IL) branch of the facial nerve, HRP-labeled neuronal cell

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bodies were seen ipsilaterally in the lateral (L) division of the facial nucleus (Fig. 1b-d).

In 3 monkeys which were injected with HRP into the orbicularis oculi muscles, HRP-labeled neuronal cell bodies were distributed in the dorsal (D) division of the facial nucleus ipsilateral to the HRP injection.

The results which were obtained from the 18 monkeys described above are summarized in Fig. 1, which shows the representation of the 5 main peripheral branches of the facial nerve within the facial nucleus of the Japanese monkey.

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### 2. Rabbit

In all rabbits examined in the present study, 6 main peripheral branches of the facial nerve were identified. These were the cervical (Ce), posterior auricular (PA), anterior auricular (AA), zygomatico-orbital (ZO), superior labial (SL), inferior labial (IL) branches of the facial nerve (Fig. 2a). The zygomatico-orbital branches (ZO) were composed of several branchlets distributing the perioral region. The perioral region was usually supplied with 4 branchlets of the facial nerve arising from the superior labial (SL) and/or the inferior labial (IL) branch of the facial nerve (Figs. 2a and 3a).

In 2 rabbits in which HRP was applied to the trunk of the facial nerve, the facial nucleus was filled with neuronal cell bodies labeled retrogradely with HRP on the side ipsilateral to the HRP application (Fig. 5a-c). No HRP-labeled neurons were found in the facial nucleus on the side contralateral to the HRP application. The facial nucleus filled with HRP-labeled motoneurons was divided into 5 divisions; into the ventromedial (V), medial (M), dorsal (D), lateral (L), and intermediate (I) divisions (Figs. 2, 3, and 5).

The HRP application was made to the cervical (Ce) branch of the facial nerve in 4 rabbits. HRP-labeled facial motoneurons were localized in the ventromedial (V) division of the facial

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nucleus ipsilateral to the HRP application (Figs. 2b and 6a). In other 4 rabbits which were injected with HRP into the superficial facial muscles around the periorbital region after cutting and ligating the superior labial (SL) and the inferior labial (IL) branches, HRP-labeled neuronal cell bodies in the facial nucleus ipsilateral to the HRP injection were located in the dorsal (D) division of the facial nucleus (Figs. 2b and 6b).

In 4 rabbits which were applied with HRP to the anterior auricular (AA) branch of the facial nerve, HRP-labeled neuronal cell bodies were seen in the dorsal (D) division of the facial nucleus, and in the dorsal part of medial (M) division of the facial nucleus ipsilateral to the HRP application (Figs. 2b and 6c).

In 3 rabbits which were applied with HRP to the posterior auricular (PA) branch of the facial nerve, HRP-labeled neuronal cell bodies were observed in the medial (M) division of the facial nucleus ipsilateral to the HRP application (Fig. 2b and 6d).

In other 3 rabbits which were applied with HRP to the superior labial (SL) branch of the facial nerve after cutting and ligating the zygomatico-orbital (ZO) and the inferior labial (IL) branches of the facial nerve, HRP-labeled neuronal cell bodies were distributed in the lateral (L) division, and in the dorsal aspect of the intermediate (I) division of the facial nucleus

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ipsilateral to the HRP application (Figs. 2b and 6e).

In 3 rabbits which were applied with HRP to the inferior labial (IL) branch of the facial nerve after cutting and ligating the superior labial (SL) and the cervical (Ce) branches of the facial nerve, HRP-labeled neuronal cell bodies were seen in the lateral (L) and the intermediate (I) divisions of the facial nucleus ipsilateral to the HRP application (Figs. 2b and 6f).

The results described above indicated that the area of the nuclear representation of the superior labial (SL) branch of the facial nerve highly overlapped with that of the inferior labial (IL) branch of the facial nerve (Fig. 2b. compare Fig. 6e with Fig. 6f). In fact, the perioral regions were usually supplied with 4 nerve branchlets arising from the superior labial (SL) and/or the inferior labial (IL) branches of the facial nerve (Figs. 2a and 3a). The 4 perioral nerve branchlets mainly supplied to the superior labial, oral angular, inferior labial and submandibular regions, respectively (Figs. 2a and 3a).

In additional 12 rabbits, the HRP application was further attempted to each of the 4 perioral nerve branchlets of the facial nerve. The 12 rabbits were divided into 4 groups and the rabbits of each group was applied with HRP to one of the perioral nerve branchlets 1, 2, 3, and 4 after cutting and ligating the other 3 perioral branchlets of the facial nerve (Fig. 3a). Facial motoneurons which were retrogradely labeled with HRP applied to

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the perioral nerve branchlet 1, 2, 3 or 4 of the facial nerve were distributed mainly in the lateral (L) division, in the dorsal part of the intermediate (I) division, intermediate part of the intermediate (I) division, or in the ventral part of the intermediate (I) division of the facial nucleus, respectively (Figs. 3 and 7).

The results obtained from the 35 rabbits described above are summarized in Figs. 2 and 3, which show the representation of peripheral nerve branches of the facial nerve within the facial nucleus.

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### **IV. DISCUSSION**

#### 1. Methodological consideration

The myotopical arrangement of facial motoneurons in the facial nucleus has been studied by the retrograde HRP method after injecting HRP into the superficial facial muscles in the opossum (Dom and Zielinsky, 1977; Provis, 1977), bat (Friauf and Herbert, 1985), mouse (Aschwell, 1982; Komiyama et al., 1984), rat (Watson et al., 1982; Hinrichsen and Watson, 1984; Friauf and Herbert, 1985), kitten (Radpour et al., 1977), cat (Kume et al., 1978; Parnes et al., 1982), and rhesus and cynomologus monkeys (Parnes et al., 1982). HRP injection into the muscle, however, has the inherent problem of leaking out of injected HRP into the surroundings. In order to avoid this inherent problem, HRP has been applied to the proximal cut-end of the peripheral branches of the facial nerve, and the representation of the main peripheral branches of the facial nerve within the facial nucleus has been investigated in the opossum (Dom, 1982), rat (Semba, 1984), guinea pig (Uemura-Sumi et al., 1986), and rabbit (Baisden et al., 1987). In the present study, the representation of the main peripheral branches of the facial nerve within the facial nucleus of the Japanese monkey and the rabbit was examined after

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application of HRP to the proximal cut-end of the target branch of the facial nerve; when HRP was injected into the superficial facial muscles, HRP was injected in the target muscle only after cutting and ligating the nerve branches supplying the superficial facial muscles adjacent to the target muscle.

The fixation of tissue in the HRP method is usually performed with a mixture of 1% paraformaldehyde and 1.25% glutaraldehyde (cf. Mesulam, 1982). In the present study, however, the fixation of tissue was done with 9% formalin in 0.1 M phosphate buffer (pH 7.3). The tissue sections fixed with 9% formalin could be counterstained more satisfactorily than those fixed with a mixture of 1% paraformaldehyde and 1.25% glutaraldehyde (cf. Itoh and Mizuno, 1977); 9% formalin in 0.1 M phosphate buffer (pH 7.3) did not seem to depress the HRP reaction, if the perfusion with 9% formalin in 0.1 M phosphate buffer (pH 7.3) is followed the perfusion with 10% sucrose in the same buffer.

### 2. Somatotopic organization of the facial nucleus

A correlation of the main peripheral branches of the facial nerve with cytoarchitectonic divisions of the facial nucleus was clearly shown by the retrograde HRP method firstly in the cat (Kume et al., 1978). Kume et al. (1978) correlated the 6 main peripheral branches of the facial nerve with the 6 cytoarchitectonic divisions of the facial nucleus. In the present study, a correlation of the 5 main peripheral branches of the facial nerve with the 5 cytoarchitectonic divisions of the facial nucleus was revealed in the Japanese monkey (Fig. 1), although it was not so clear-cut as reported in the cat by Kume et al. (1978): The inferior labial, the cervical, or the posterior auricular branch of the facial nerve was represented in the lateral, the ventral, or the medial division of the facial nucleus, respectively. The auriculo-zygomatico-orbital branch of the facial nerve was represented in the intermediate and the dorsal divisions of the facial nucleus. The superior labial branch of the facial nerve was represented in the dorsal and lateral division of the facial nucleus.

Thus, it was indicated in the Japanese monkey that the ventral, the medial, or the intermediate division of the facial nucleus supplied the cervical, the posterior auricular, or the auriculo-zygomatico-orbital branch of the facial nerve,

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respectively; the dorsal division of the facial nucleus supplied the auriculo-zygomatico-orbital and the superior labial branches of the facial nerve; the lateral division of the facial nucleus supplied the superior labial and the inferior labial branches of the facial nerve.

The present results obtained from the rabbits confirmed and extended those reported by Baisden et al. (1987). In the rabbit. main peripheral branches of 6 the facial nerve. and 5 cytoarchitectonic divisions of the facial nucleus were identified (Figs. 2 and 3). The cervical, the posterior auricular, or the zygomatico-orbital branch of the facial nerve was represented in the ventromedial, the medial, or the dorsal division of the facial nucleus, respectively. The anterior auricular branch of the facial nerve was represented in the medial and the dorsal divisions of the facial nucleus. Both the superior labial and the inferior labial branches of the facial nerve were represented in the lateral and the intermediate divisions of the facial nucleus.

Thus, it was indicated in the rabbit that the ventromedial division of the facial nucleus supplied the cervical branch of the facial nerve; the medial division of the facial nucleus supplied the anterior auricular and the posterior auricular branches of the facial nerve; both the intermediate and the lateral divisions of the facial nucleus supplied the superior

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labial and the inferior labial branches of the facial nerve; the dorsal division of the facial nucleus supplied the anterior auricular and the zygomatico-orbital branches of the facial nerve.

The correlation of cytoarchitectonic divisions of the facial nucleus with the main peripheral branches of the facial nerve was not so clear-cut in the rabbit as reported in the cat by Kume et al. (1978). Overlapping of the origins of the main peripheral branches of the facial nerve within a cytoarchitectonic division of the facial nucleus as seen in the present study has also been reported in the rat (Semba 1984), guinea pig (Uemura-Sumi et al., 1986), and rabbit (Baisden et al., 1987).

The area of distribution of facial motoneurons supplying the perioral region appeared to occupy a larger proportion of the nuclear region in the rabbit than in the monkey. On the other hand, the area of distribution of facial motoneurons supplying the periorbital region appeared to occupy a smaller proportion of the nuclear region in the rabbit than in the monkey.

The present results, in combination with those of the previous studies in a variety of mammals (for references, cf. INTRODUCTION), suggest that species-differences are more or less present in both branching pattern of the facial nerve and cytoarchitecture of the facial nucleus. On the other hand, it is also indicated that there exists a common principle in the

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somatotopic organization of the mammalian facial nucleus; the cervical, auricular, periorbital, or perioral region of the face is represented in the ventral, medial, dorsal, or lateral aspect of the facial nucleus, respectively.

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#### V. SUMMARY

In an attempt to throw more light on the general plan of somatotopic organization of the facial nucleus in mammalian species, correlation of the main peripheral branches of the facial nerve with the cytoarchitectonic divisions of the facial nucleus was examined in the Japanese monkey (<u>Macaca fuscata</u>) and the rabbit by the tracing technique of retrograde axonal transport of horseradish peroxidase (HRP). The results are summarized diagrammatically in Figs. 1, 2, and 3.

In the monkey, 5 main peripheral branches of the facial nerve were identified; they were the cervical (Ce), posterior auricular (PA), auriculo-zygomatico-orbital (AZO), superior labial (SL), and inferior labial (IL) branches. The facial nucleus was divided cytoarchitectonically into 5 divisions; they were the ventral (V), medial (M), intermediate (I), dorsal (D), and lateral (L) divisions. When HRP was applied to the cervical (Ce), posterior auricular (PA), or inferior labial (IL) branch of the facial nerve, neuronal cell bodies which were retrogradely labeled with HRP were seen respectively in the ventral (V), medial (M), or lateral (L) division of the facial nucleus ipsilateral to the HRP application. When HRP was applied to the auriculo-zygomatico-orbital (AZO) branch of the facial nerve, HRP-labeled neuronal cell bodies were observed mainly in the

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intermediate (I) and dorsal (D) divisions of the facial nucleus ipsilateral to the HRP application. When HRP was applied to the superior labial (SL) branch of the facial nerve, HRP-labeled neuronal cell bodies were seen in the dorsal (D) and lateral (L) divisions of the facial nucleus ipsilateral to the HRP application.

Thus, it was indicated that the ventral (V), medial (M), or intermediate (I) division of the facial nucleus contained motoneurons supplying the cervical (Ce), posterior auricular (PA), or auriculo-zygomatico-orbital (AZO) branch of the facial nerve, respectively; the dorsal (D) division of the facial nucleus were distributed with motoneurons supplying the auriculo-zygomatico-orbital (AZO) and superior labial (SL) branches of the facial nerve; the lateral (L) division of the facial nucleus contained motoneurons supplying the superior labial (SL) and inferior labial (IL) branches of the facial nerve.

In the rabbit, 6 main peripheral branches of the facial nerve were identified; they were the cervical (Ce), posterior auricular (PA), anterior auricular (AA), zygomatico-orbital (ZO), superior labial (SL), and inferior labial (IL) branches of the facial nerve. The facial nucleus was divided into 5 cytoarchitectonic divisions; they were the ventromedial (V), medial (M), intermediate (I), dorsal (D), and lateral (L)

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divisions. The results obtained by the retrograde HRP method indicated that the ventromedial division of the facial nucleus contained motoneurons supplying the cervical branch of the facial nerve; the medial division of the facial nucleus supplied the anterior auricular and the posterior auricular branch of the facial nerve; the dorsal division of the facial nucleus was relatively small and contained motoneurons innervating the superficial facial muscles in the periorbital region through the anterior auricular and the zygomatico-orbital branches of the facial nerve; the intermediate and the lateral divisions of the facial nucleus were distributed with many motoneurons innervating the superficial facial muscles in the perioral region through the anterior auricular and the inferior labial branches of the facial nerve.

The present results, in combination with those of the previous studies, suggest that in spite of the presence of species-differences in the branching pattern of the facial nerve and the cytoarchitectonic organization of the facial nucleus, a common principle exists in the somatotopic organization of the mammalian facial nucleus; the cervical, auricular, periorbital, or perioral region of the face is represented in the ventral, medial, dorsal, or lateral aspect of the facial nucleus, respectively.

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#### **VII. REFERENCES**

Ashwell, K. W. (1982) The adult mouse facial nerve nucleus: Morphology and musculotopic organization. J. Anat., 135: 531-538.

- Baisden, R. H., M. L. Woodruff, D. L. Whittington, D. C. Baker, and A. E. Benson (1987) Cells of origin of the branches of the facial nerve: A retrograde HRP study in the rabbit. Am. J. Anat., 178: 175-184.
- Courville, J. (1966) The nucleus of the facial nerve: The relation between cellular groups and peripheral branches of the nerve. Brain Res., 1: 338-354.
- Dom, R. M. (1982) Topographical representation of the peripheral nerve branches of the facial nucleus of the opossum: A study utilizing horseradish peroxidase. Brain Res., 246: 281-284.
  Dom, R. M., and X. J. Zielinski (1977) Major subdivisions of the facial nucleus of the pouch young opossum, Didelphis

marsupialis virginiana. Anat. Rec., 187: 567 (abstract).

Dom, R. M., W. Falls, and G. F. Martin (1973) The motor nucleus of the facial nerve in the opossum (<u>Didelphis marsupialis</u> <u>virginiana</u>). Its organization and connections. J. Comp. Neurol., 152: 373-402.

- 29 -

- Friauf, E., and H. Herbert (1985) Topographic organization of facial motoneurons to individual pinna muscles in rat (<u>Rattus</u> <u>rattus</u>) and bat (<u>Rousettus</u> <u>aegyptiacus</u>). J. Comp. Neurol., 240: 161-170.
- Hinrichsen, C. F. L., and C. D. Watson (1984) The facial nucleus of the rat: Representation of facial muscles revealed by retrograde transport of horseradish peroxidase. Anat. Rec., 209: 407-415.
- Itoh, K., and N. Mizuno (1977) Topographical arrangement of thalamocortical neurons in the centrolateral nucleus (CL) of the cat, with special reference to a spino-thalamo-motor cortical path through the CL. Exp. Brain Res., 30: 471-480.Kitai, S. T., T. Tanaka, N. Tsukahara, and H. Yu (1972) The facial nucleus of cat: Antidromic and synaptic activation and peripheral nerve representation. Exp. Brain Res., 16: 161-183.
- Komiyama, M., H. Shibata, and T. Suzuki (1984) Somatotopic representation of facial muscles within the facial nucleus of the mouse. A study using the retrograde horseradish peroxidase and cell degeneration techniques. Brain Behav. Evol., 24: 144-151.

- 30 -

- Kume, M., M. Uemura, K. Matsuda, R. Matsushima, and N. Mizuno (1978) Topographical representation of peripheral branches of the facial nerve within the facial nucleus: A HRP study in the cat. Neurosci. Lett., 8: 5-8.
- Martin, M. R., and D. Lodge (1977) Morphology of the facial nucleus of the rat. Brain Res., 123: 1-12.
- Mesulam, M-M. (1978) Tetramethyl benzidine for horseradish peroxidase neurohistochemistry: A non-carcinogenic blue reaction-product with superior sensitivity for visualizing neural afferents and efferents. J. Histochem. Cytochem., 26: 106-117.
- Mesulam, M-M. (1982) Principles of horseradish peroxidase neurohistochemistry and their applications for tracing neural pathways - Axonal transport, enzyme histochemistry and light microscopic analysis. In: Mesulam, M-M. (ed.) Tracing Neural Connections with Horseradish Peroxidase. Wiley, New York, pp. 1-151.
- Mizuno, N., A. Konishi, and M. Sato (1975) Localization of masticatory motoneurons in the cat and rat by means of retrograde axonal transport of horseradish peroxidase. J. Comp. Neurol., 164: 105-116.

Papez, J. W. (1927) Subdivisions of the facial nucleus. J. Comp. Neurol., 43: 159-191.

- 31 -

- Pappas, P. W. (1971) The use of a chrome alum-gelatin (subbing) solution as a general adhesive for paraffin sections. Stain Technol., 46: 121-124.
- Parnes, S. M., N. Strominger, S. Silver, and J. C. Goldstein (1982) Alternate innervations of facial musculature. Arch. Otolaryngol., 108: 418-421.
- Provis, J. (1977) The organization of the facial nucleus of the brush-tailed possum (<u>Trichosurus vulpecula</u>). J. Comp. Neurol., 172: 177-188.
- Radpour, S. (1977) Organization of the facial nerve nucleus in the cat. Laryngoscope 87: 557-574.
- Semba, K. (1984) Topographical representation of peripheral branches of the facial nerve in the facial motor nucleus revealed by horseradish peroxidase transport in the rat. Anat. Rec., 208: 162A-163A (abstract).
- Shohara, E., and A. Sakai (1983) Localization of motoneurons innervating deep and superficial facial muscles in the rat: A horseradish peroxidase and electrophysiologic study. Exp. Neurol., 81: 14-33.
- Szentágothai, J. (1948) The representation of facial and scalp muscles in the facial nucleus. J. Comp. Neurol., 88: 207-220.

- 32 -

Uemura-Sumi, M., Y. Manabe, R. Matsushima, and N. Mizuno (1986) Correlation of the main peripheral branches of the facial nerve with the cytoarchitectonic subdivisions of the facial nucleus in the guinea pig. Anat. Embryol., 174: 161-166. Vraa-Jensen, G. (1942) The Motor Nucleus of the Facial Nerve.

With a Survey of the Efferent Innervation of the Facial Muscles. A Normal-Anatomical Study. Munksgaard, Saabye & Christensen, Copenhagen, pp. 157.

Watson, C. R. R., S. Sakai, and W. Armstrong (1982) Organization of the facial nucleus in the rat. Brain Behav. Evol., 20: 19-28.

Yagita, K. (1910) Experimentelle Untersuchungen über den Ursprung des Nervus facialis. Anat. Anz., 38: 195-218. Fig. 1. The pattern of branching of the facial nerve (a), and the representation of the 5 main peripheral branches of the facial nerve within the facial nucleus (b-d) in the Japanese monkey.

a: The sites of HRP application are indicated with double lines crossing the cervical (Ce), posterior auricular (PA), auriculo-zygomatico-orbital (AZO), superior labial (SL), and inferior labial (IL) branches of the facial nerve. AA, nerve branchlets supplying the anterior auricular region; ZO, nerve branchlets innervating the zygomatico-orbital region.

b-d: Within 3 cross-sections through a rostral (b), a middle (c), and a caudal (C) level of the facial nucleus, the pattern of distribution of facial motoneurons which were labeled with HRP applied to the main peripheral branches of the facial nerve is shown diagrammatically. Facial motoneurons supplying the Ce, PA, AZO, SL, or IL branch of the facial nerve are indicated with filled squares, asterisks, filled triangles, crosses, or filled circles, respectively. The facial nucleus is divided into the ventral (V), medial (M), intermediate (I), dorsal (D), and lateral (L) divisions. The ventral (V) division is further divided into 2 subdivisions at the middle (c) and caudal (d) levels of the facial nucleus.



Fig. 2. The pattern of branching of the 6 main peripheral branches of the facial nerve (a), and the representation of them within the facial nucleus (b) in the rabbit.

a: The sites of HRP application are indicated with double lines crossing the cervical (Ce), anterior auricular (AA), posterior auricular (PA), superior labial (SL), and inferior labial (IL) branches of the facial nerve. ZO, zygomatico-orbital branch of the facial nerve.

b: Within 3 cross-sections through a rostral, a middle, and a caudal level of the facial nucleus, the pattern of distribution of facial motoneurons which were labeled with HRP applied to the main peripheral branches of the facial nerve is shown diagrammatically. Facial motoneurons supplying the Ce, ZO, AA, PA, SL, or IL branch of the facial nerve are indicated with filled squares, filled triangles, open triangles, filled stars, crosses, or filled circles, respectively. The facial nucleus is divided into the ventromedial (V), medial (M), dorsal (D), lateral (L), and intermediate (I) divisions. Dor, dorsal; Lat, lateral.



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Fig. 3. The pattern of representation of the 4 perioral branchlets of the facial nerve within the facial nucleus of the rabbit.

a: The sites of HRP application are indicated with the double lines crossing the 4 perioral branchlets (1-4) of the facial nerve.

b: Within a cross-section through a middle level of the facial nucleus, the pattern of distribution of facial motoneurons which were labeled with HRP applied to the 4 perioral branchlets of the facial nerve is shown diagrammatically. Facial motoneurons supplying the branchlet 1, 2, 3, or 4 are indicated with crosses, open triangles, filled circles, or filled stars, respectively. Abbreviations are as in Fig. 2.



Fig. 4. Photomicrographs of 4 cross-sections through the middle levels of the facial nucleus of the Japanese monkey, showing distribution of facial motoneurons which were labeled with HRP applied to the trunk of the facial nerve (a), cervical branch (b), posterior auricular branch (c), or superior labial branch (d) of the facial nerve. HRP-labeled neurons are seen in the all divisions (a), ventral division(b), medial division (c), or dorsal and lateral divisions (d) of the facial nucleus. HRP-labeled neurons in the ventral division are clustered into 2 subgroups (b). Scale = 200  $\mu$ m. Abbreviations are as in Fig. 1.



Fig. 5. a-c: Photomicrographs of 3 cross-sections through a rostral (a), a middle (b), and a caudal (c) level of the facial nucleus of the rabbit, showing retrograde labeling of facial motoneurons after HRP application to the trunk of the facial nerve. Scale =  $200 \ \mu m$ .

d: The cytoarchitectonic divisions at a middle level of the facial nucleus of the rabbit are shown diagrammatically. Abbreviations are as in Fig. 2.



Fig. 6. Photomicrographs of 6 cross-sections through the middle levels of the facial nucleus of the rabbit, showing distribution of facial motoneurons which were labeled with HRP applied to the cervical (a), zygomatico-orbital (b), anterior auricular (c), posterior auricular (d), superior labial (e), or inferior labial (f) branch of the facial nerve. Scale = 200  $\mu$ m. Abbreviations are as in Fig. 2.



Fig. 7. Photomicrographs of 4 cross-sections through the middle levels of the facial nucleus of the rabbit, showing the distribution of facial motoneurons which were labeled with HRP applied to the perioral branchlet 1 (a), 2 (b), 3 (c), or 4 (d) of the facial nerve (cf. Fig. 3). Scale = 200  $\mu$ m. Abbreviations are as in Fig. 2.

