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The adult mouse facial nerve nucleus: morphology and musculotopic organization

K. W. ASHWELL

School of Anatomy, University of New South Wales, Kensington, New South Wales

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INTRODUCTION

The representation of the facial musculature within the facial nucleus has been demonstrated for a wide variety of animals including the rat (Papez, 1927; Watson & Sakai, 1978), the cat (Courville, 1966; Kume *et al.* 1978; Papez, 1927), the brush-tailed possum (Provis, 1977) and the opossum (Dom & Zeilinski, 1977; Papez, 1927). The present paper describes the morphological arrangement of the mouse facial nucleus and also the musculotopic organization as revealed by the horseradish peroxidase (HRP) technique. The study was made in order to compare the musculotopic organization of the mouse facial nucleus with that of other mammals and also to examine the possible relationship of nuclear topography to the characteristic whisking behaviour of mice.

MATERIALS AND METHODS

All animals used in this study were BALB/c strain mice. The form of the nucleus of the facial nerve was studied in serial, Nissl-stained, 50 μm frozen sections from three animals, the identification of the facial nucleus having been confirmed by reference to an atlas (Sidman, Angevine & Taber-Pierce, 1971). Facial motoneurons were easily identifiable by their size and prominent Nissl substance. Neuronal counts were performed by counting all neuronal nuclei in the above Nissl-stained sections and correcting for double counting according to Abercrombie (1946). Note that both sides of only one animal were counted.

Animals for the 28 HRP experiments were anaesthetised with ether or pentobarbitone (35 mg/kg) and injected in various parts of the facial musculature with volumes of 2·5–10 μl HRP solution (10, 25 and 40 % w/v) made by dissolving HRP (Boehringer–Mannheim) in 0·1 M tris buffered to pH 7·6 with HCl. Table 1 shows the number of experiments for each muscle or muscle group. Figure 1 A shows the superficial injection sites. In the case of the superficial muscles, injection of the individual fine muscles in the nasolabial region and around the auricles (as described by Meble & Stromberg, 1976, for the rat) was not feasible. Instead these thin, delicate muscles were grouped together for the purposes of this study (e.g. nasolabial musculature, anterior auricular musculature) and subcutaneous injections were made into the region as a whole. In the case of the posterior belly of the digastric muscle, the injections were made directly into the muscle belly following surgical exposure. Injections were also made through the tympanic membrane, depositing HRP in the middle ear in an attempt to label those motoneurons supplying the stapedius muscle.

Table 1. *The number of experiments performed for each injection site*

Injection site	No. of experiments
Nasolabial musculature	6
Orbicularis oculi muscle	4
Anterior auricular musculature	4
Posterior auricular musculature	7
Posterior digastric muscle	2
Mentalis/platysma muscles	3
Stapedius muscle	2

All animals were allowed to survive for 24 hours. They were then deeply anaesthetised with pentobarbitone sodium and perfused through the heart with normal saline followed by a fixative composed of 1 % paraformaldehyde and 2·5 % glutaraldehyde in 0·1 M phosphate buffer (pH 7·5). The brains were removed and placed overnight in phosphate-buffered (0·1 M, pH 7·5) 30 % sucrose solution. The brain stems were sectioned at 50 μm on a freezing microtome and the sections were reacted for the demonstration of HRP by the Mesulam (1978) technique. The sections were counterstained with neutral red.

Unfortunately, due to the difficulty of decalcifying the head of the mouse without destroying HRP activity, it was not possible to determine histologically the extent of HRP diffusion from the injected muscle to adjacent muscles. Instead, a variety of volumes of HRP was injected into several animals and the musculotopic organization of the nucleus was determined by noting the recession of HRP labelling into various parts of the nucleus as the volumes injected were reduced.

Three animals for facial muscle volume determinations were deeply anaesthetised with pentobarbitone sodium and perfused through the heart with normal saline followed by 10 % formalin in normal saline. They were then decapitated and the heads were placed in a decalcifying solution (made by mixing saturated aqueous picric acid, concentrated formalin and 92 % formic acid in the ratios 1:1:1). The heads were changed to fresh solution every 2–3 days and decalcification was complete in 3–4 weeks. The heads were sectioned in the coronal plane on a freezing microtome at 200 or 500 μm. One section was collected every millimetre and mounted unstained and undehydrated, under Hydramount (a water mounting medium). Volumes of various facial muscles were determined from these sections, using an eyepiece grid with squares calibrated to a standard area. The number of squares in the grid subtended by a muscle was counted and this was converted to μm^2 to obtain the muscle area in each section. This value was then multiplied by the inter-section distance. The volume of any facial muscle was then taken as the sum of these volumes for all sections in which the muscle appeared.

Because the aim of the muscle volume measurements was to correlate the nasolabial musculature volume with HRP labelling in the nucleus, the small superficial facial muscles were grouped together as described previously.

RESULTS

Nuclear morphology

The facial nucleus was found to be divided into seven subnuclei, best seen in coronal section. Six of these subnuclei lay within the main body of the facial motor

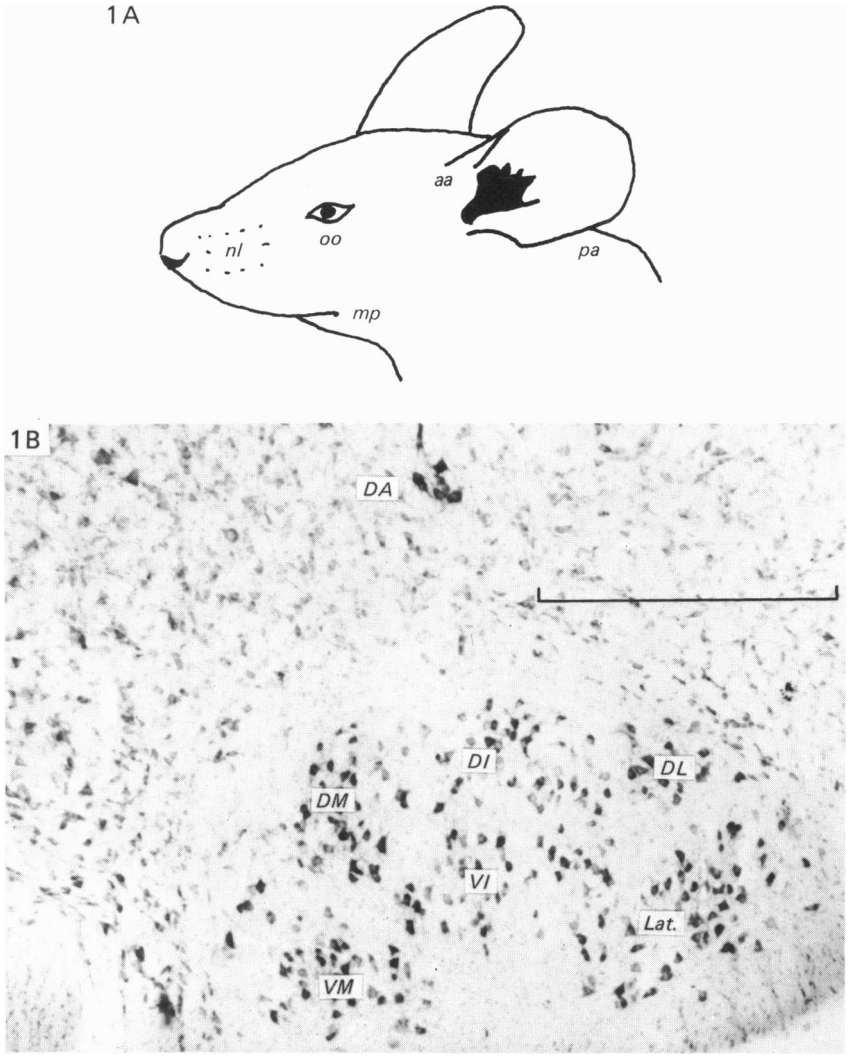

Fig. 1(A). The sites into which subcutaneous HRP injections were made: *nl*, nasolabialis musculature; *oo*, orbicularis oculi muscle; *aa*, anterior auricular musculature; *pa*, posterior auricular musculature; *mp*, mentalis and associated portions of platysma. Note that the stapedius muscle and the posterior belly of the digastric muscle are not shown on this diagram. (B) The subnuclear organization of the mouse facial nerve nucleus seen in a coronal section through the rostral part of the nucleus. Letters indicate the names of subnuclei: *Lat*, lateral; *DL*, dorsolateral; *DI*, dorsal intermediate; *VI*, ventral intermediate; *DM*, dorsomedial; *VM*, ventromedial; *DA*, dorsal accessory. Bar indicates 0·5 μm.

nucleus, while the seventh, the dorsal accessory, lay in a position slightly dorsal to the rostral portion of the main nucleus. The relative position of each of the various subnuclei is shown in Figure 1B.

There was an average of 2027 motoneurons in the facial nucleus from counts of two sides of one adult mouse. This agrees closely with results obtained from a day 16 postnatal mouse (2070) and 2 day 10 postnatal mice (2049; 2182). A report of a detailed analysis of the development of the mouse facial nucleus is in preparation. The counts for each subnucleus of the facial nucleus are shown in Table 2.

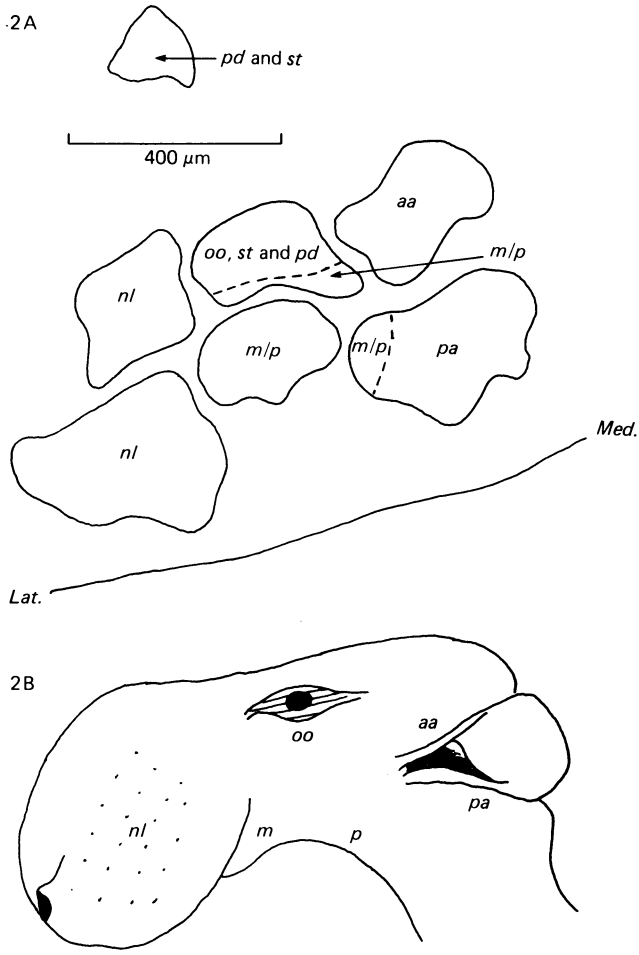

Fig. 2 (A). The musculotopic organization of the adult mouse facial nucleus. Nuclear map in coronal section with muscles suppplied by neurons lying in particular subnuclei shown by lower case letters in the relevant regions of the nucleus. *nl*, nasolabial musculature: *oo*, orbicularis oculi muscle; *m/p*, mentalis and associated portions of platysma; *aa*, anterior auricular musculature; *pa*, posterior auricular musculature; *pd*, posterior belly of the digastric muscle; *st*, stapedius muscle. (B) Caricature of a mouse obtained by distorting a lateral view of the head so that regions of the face are given areas corresponding to the sizes of the portions of the nucleus which supply them. Muscles injected are shown by lower case letters as for (A). Note that the stapedius and digastric muscles have been omitted from this diagram to minimise confusion.

HRP data

The musculotopic organization, as revealed by 28 experiments, is summarized in Figure 2. Figure 3 shows photographs of two typical experiments, while Figure 4 shows a high power view of HRP-labelled motoneurons.

DISCUSSION

Neuronal numbers

The value obtained in this study for the number of motoneurons within the facial nucleus disagrees with the results of Shimozawa (1975), who found an average of

Table 2. *The number of motoneurons in the adult mouse facial nerve nucleus*
(Columns show corrected counts for both sides of one animal, mean of the two sides and the mean number for each subnucleus expressed as a percentage of the total mean.)

Subnucleus	Left	Right	Mean	Percentage of total mean
Lateral	698	677	687·5	34·0
Dorsolateral	186	180	183	9·0
Ventromedial	392	329	360·5	17·8
Dorsomedial	192	253	222·5	10·9
Dorsal intermediate	399	353	376	18·6
Ventral intermediate	174	171	172·5	8·5
Dorsal accessory	22	27	24·5	1·2
Total	2063	1990	2026·5	

3215 large myelinated fibres in the motor root of the adult mouse facial nerve. This discrepancy could possibly be explained by genetic differences, but Shimozawa does not report the strain of mice used in his study. Alternatively, some of the large myelinated fibres in the facial nerve motor root may not be destined for facial muscles, or some facial motoneuron axons may have collaterals.

The musculotopic organization of the facial nucleus

The present data concerning the musculotopic organization of the facial nucleus in the mouse are in general agreement with findings in other animals; the lateral portions of the nucleus supply facial musculature in the nasolabial regions and the medial portions supply the auricular musculature (Courville, 1966; Papez, 1927; Provis, 1977; Watson & Sakai, 1978). In the present study, motoneurons innervating the stapedius muscle were found only in the dorsal regions of the nucleus, particularly in the dorsal accessory and dorsal intermediate subnuclei. This corresponds well with Vraa Jensen's (1942) finding in the dog, although Lyon (1978) in the cat found that most of the motoneurons supplying the stapedius muscle were located between the caudal end of the lateral superior olivary nucleus and the facial nucleus.

It will be noted that the nasolabial musculature is represented by a relatively large portion of the facial nucleus – the lateral and dorsolateral subnuclei. From the cell counts it is seen that these subnuclei contain approximately 43 % of all motoneurons in the facial nucleus. Measurements of the facial muscle volume revealed that the relatively large representation of the nasolabial musculature in the facial nucleus can be attributed to the large volume of these muscles (approximately 40 % of total facial musculature volume). Thus there is no evidence of a dense innervation of these muscles as compared to the auricular musculature.

The relative size of the representation of the nasolabial musculature within the facial nucleus is of interest, because these muscles are important in whisking behaviour in rodents (Welker, 1964). Watson & Saki (1978) noted that, in the rat, the nasolabial musculature appeared to be represented by a relatively small portion of the facial nucleus, considering the importance of whisking behaviour to rodents. This might also apply to the mouse, because the percentage of all facial motoneurons which lie within those subnuclei responsible for innervation of the nasolabial musculature is similar to the percentage of total facial muscle volume found for the nasolabial musculature. This suggests that the density of innervation of these

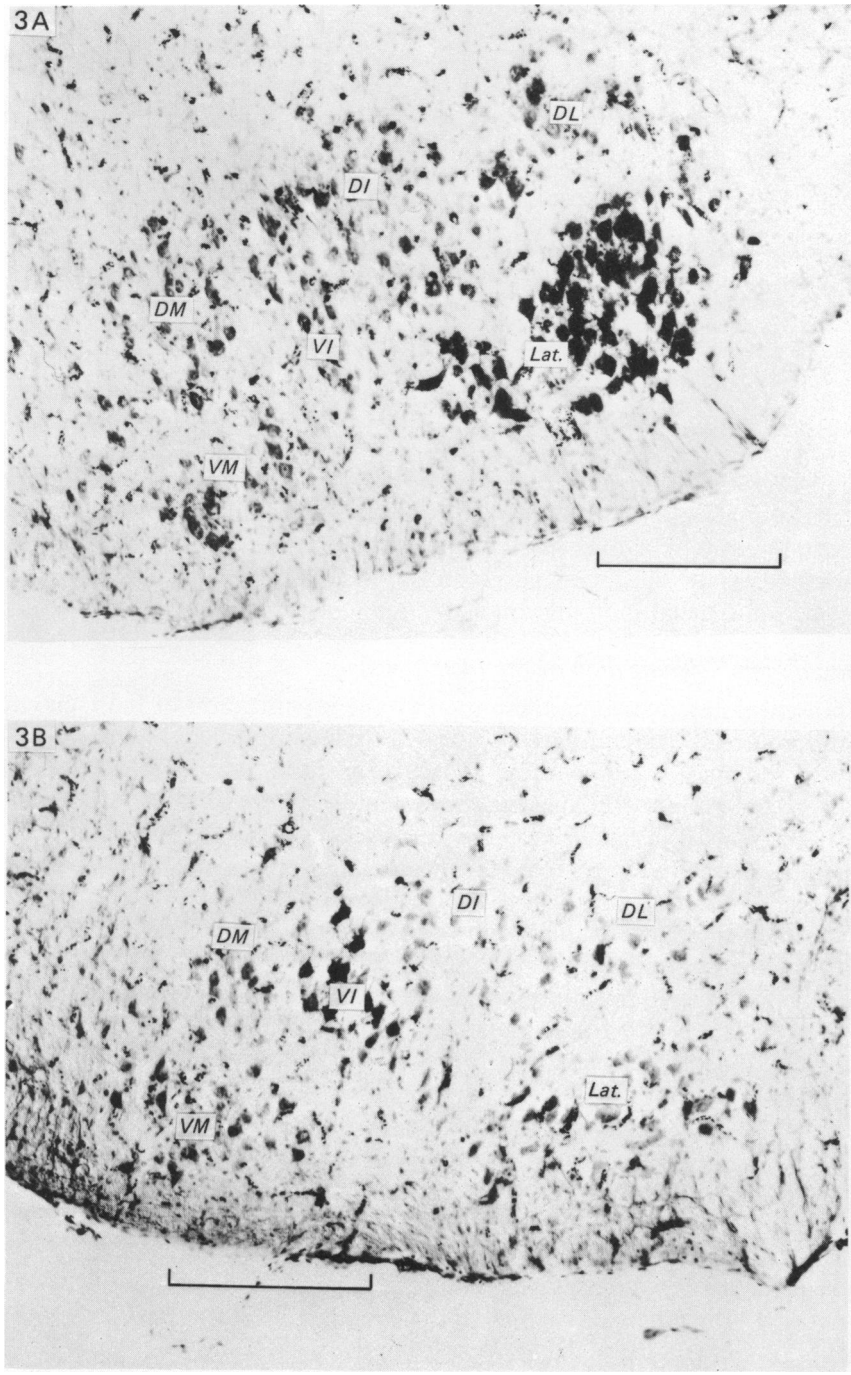

Fig. 3. HRP labelling of facial motoneurons in two typical experiments. Bars indicate 200 μm. (A) Labelling following a small injection in the nasolabial region. Subnuclei are indicated as for Fig. 1. Labelled motoneurons are seen in the lateral subnucleus with some in the dorsolateral subnucleus. (B) Labelling following injection in the mentalis/platysma region. Labelled motoneurons are seen mainly in the ventral intermediate subnucleus.

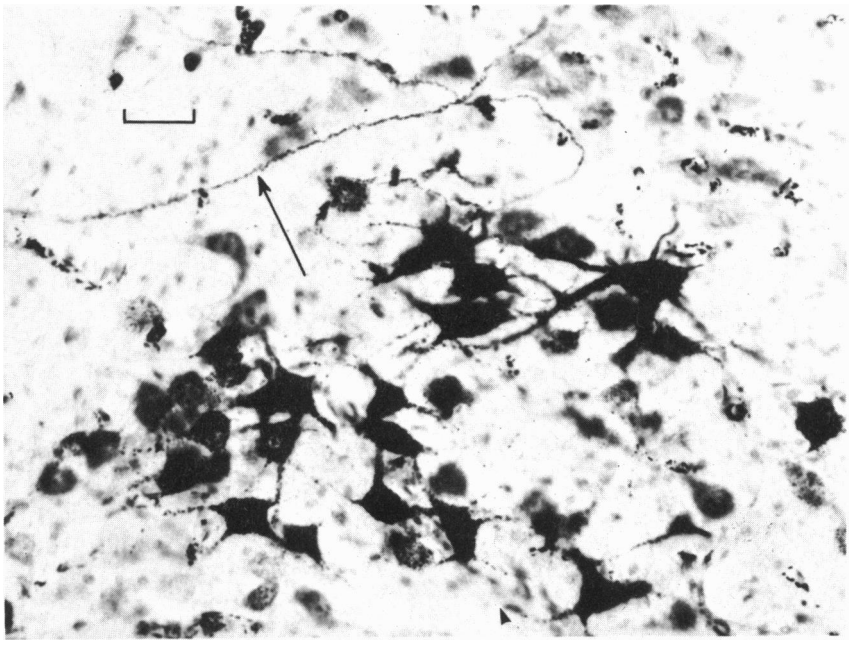

Fig. 4. High power view of labelled facial motoneurons in the ventro medial subnucleus following HRP injection in the posterior auricular region. Arrow indicates labelled motoneuron axon. Bar indicates 20 μm.

nasolabial muscles lies close to the average for all facial muscles, and is not higher, as might be expected considering the importance of whisking behaviour. It has been suggested that the relatively unremarkable representation of the nasolabial musculature within the facial nucleus in the rat may be due to the observed stereotyped nature of whisking behaviour (Erzurumlu & Killackey, 1979) so that, despite the importance of this behaviour in rodents, the nasolabial musculature does not require a particularly dense innervation. Similarly, this could explain the unremarkable density of innervation of nasolabial musculature in the mouse.

SUMMARY

The adult mouse facial nerve nucleus was found to contain seven subnuclei, six of which lay within the main nucleus, while the seventh, the dorsal accessory, lay slightly dorsal to the main nucleus. The facial musculature was found to be represented within the facial nucleus so that those neurons supplying the nasolabial musculature lay in the lateral portions of the nucleus while the auricular musculature was innervated by motoneurons in the medial parts of the nucleus. This musculotopic organization is similar to that found for other mammalian facial nuclei.

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