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Functional Circuitry Involved in the Regulation of Whisker Movements

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Abstract

Neuroanatomical tract-tracing methods were used to identify the oligosynaptic circuitry by which the whisker representation of the motor cortex (wMCx) influences the facial motoneurons that control whisking activity (wFMNs). Injections of the retrograde tracer cholera toxin subunit B into physiologically identified wFMNs in the lateral facial nucleus resulted in dense, bilateral labeling throughout the brainstem reticular formation and in the ambiguous nucleus as well as predominantly ipsilateral labeling in the paralemniscal, pedunculopontine tegmental, Kölliker-Fuse, and parabrachial nuclei. In addition, neurons in the following midbrain regions projected to the wFMNs: superior colliculus, red nucleus, periaqueductal gray, mesencephalon, pons, and several nuclei involved in oculomotor behaviors. Injections of the anterograde tracer biotinylated dextran amine into the wMCx revealed direct projections to the brainstem reticular formation as well as multiple brainstem and midbrain structures shown to project to the wFMNs. Regions in which retrograde labeling and anterograde labeling overlap most extensively include the brainstem parvocellular, gigantocellular, intermediate, and medullary (dorsal and ventral) reticular formations; ambiguous nucleus; and midbrain superior colliculus and deep mesencephalic nucleus. Other regions that contain less dense regions of combined anterograde and retrograde labeling include the following nuclei: the interstitial nucleus of medial longitudinal fasciculus, the pontine reticular formation, and the lateral periaqueductal gray. Premotoneurons that receive dense inputs from the wMCx are likely to be important mediators of cortical regulation of whisker movements and may be a key component in a central pattern generator involved in the generation of rhythmic whisking activity.

Keywords

central pattern generator; motor cortex; facial nucleus; brainstem; rat

Production of rhythmic whisker movements (“whisking”) is a critical exploratory behavior in several mammalian species (see, e.g., Vincent, 1912; Brecht et al., 1997) and is emerging as a model system for studying mechanisms of voluntary movements (Kleinfeld et al., 1997). Whisking consists of large-amplitude protractions of the large mystacial vibrissae, with spectral components between 6 and 9 Hz (Semba and Komisaruk, 1984). The whisker representation of the motor cortex (wMCx) occupies approximately one-third of the rodent motor cortex, and low-intensity stimulation of this region evokes whisker protractions (Donoghue and Wise, 1982; Weiss and Keller, 1994). The patterns of afferent, efferent, and intracortical connections of the wMCx suggest that the area is devoted primarily to regulating whisker movements (Donoghue and Parham, 1983; Miyashita et al., 1994; Weiss and Keller, 1994). However, the mechanisms by which the wMCx regulates whisking are at present

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unknown. Support for a role of the wMCx in regulating whisking is derived from studies demonstrating correlations between wMCx unit activity and EMG recorded from the whisker pad (Carvell et al., 1996) and the ability of the whiskers to entrain rhythmic stimulation of the motor cortex at low frequencies (Kleinfeld et al., 1999). Although such correlational data are informative, they do not directly illustrate the mechanisms by which cortical outputs regulate whisking. Earlier studies showed that ablation of the entire frontal cortex “does not affect whisking” (Welker, 1964; Semba and Komisaruk, 1984). However, neither of these studies systematically analyzed the effects of these lesions on patterned whisking behaviors. Although rhythmic whisker movements remained preserved, other properties such as whisking magnitude were reduced. Indeed, we have recently demonstrated that uni-lateral lesions of the wMCx dramatically affect the kinematics of whisker movements (Keller et al., 2001). A similar result was found in the monkey after lesions of the lateral precentral cortex, which affected the magnitude of mastication but not its underlying rhythmic nature (Larson et al., 1980). This suggests that rhythmic facial movements are generated by a subcortical pattern generator (Gao et al., 2001a) whose activity is regulated by cortical inputs. The location of this central pattern generator (CPG), its connections with the wMCx and whisking facial motoneurons (wFMNs), and its functional properties are at present unknown.

The rodent facial nucleus does *not* receive direct inputs from forebrain structures (Isokawa-Akesson and Komisaruk, 1987). More specifically, we have shown that the wMCx does not project to any of the facial subnuclei (Miyashita et al., 1994). Thus, motor cortex regulation of wFMNs is likely mediated through an oligosynaptic pathway in which cortical inputs regulate premotoneurons that are part of a whisking CPG. These pre-wFMNs project to and drive the rhythmic activity of wFMNs. Indirect evidence suggests that several brainstem and mid-brain regions may be involved in these interactions. Several anatomical and physiological studies identified cortical and subcortical targets of wMCx efferents (Miyashita et al., 1994) as well as subcortical regions that project to the facial nucleus (Erzurumlu and Killackey, 1979; Hinrichsen and Watson, 1983; Fanardjian and Manvelyan, 1987; Isokawa-Akesson and Komisaruk, 1987; Li et al., 1993; Mogoseanu et al., 1994). However, insofar as the facial nucleus is a heterogeneous structure containing motoneurons that project to multiple muscle groups, these studies provide no direct information on the sources of inputs to identified wFMNs. Furthermore, except for connections via the superior colliculus (Miyashita and Mori, 1995), there is no direct evidence for the existence of wMCx→pre-FMN→wFMN pathways. The goal of the present study was to identify structures that receive direct inputs from the wMCx and provide inputs to physiologically identified wFMNs by using combined antero- and retrograde tract-tracing techniques. Some of these results have been published previously in abstract form (Hattox et al., 2000).

MATERIALS AND METHODS

All procedures were approved by the University of Maryland School of Medicine and complied with the NIH guidelines for the care and use of laboratory animals.

Surgical procedures

Ten male Sprague-Dawley rats (aged 45–55 days) were injected with the retrograde tracer cholera toxin subunit B (CTB; List Biotechnology Laboratories, Campbell, CA). Surgery was performed using sterile techniques on rats anesthetized by intramuscular injection of ketamine (100 mg/kg) and xylazine (0.2–0.4 mg). Body temperature was maintained at 37°C with a thermostatically regulated heating pad. The rats were placed in a stereotaxic device, and the lateral facial nucleus was accessed for CTB injections through an oblique dorsal approach between the posterior and the rostral portions of the cerebellum and the brainstem, with a 58° angle from the vertical. This oblique approach was used to minimize inadvertent labeling of

axons of passage in the brainstem and midbrain. A double-barrel pipette with a tip diameter of 10–20 μm was advanced approximately 4.2 mm deep to reach the facial nucleus. One barrel contained CTB, and the second was filled with NaCl and used for extracellular field recordings. We physiologically identified the lateral facial nucleus by recording extracellular field potentials evoked by electrical stimulation of individual whisker follicles. Follicles were stimulated through a unipolar metal electrode, inserted directly into an individual follicle, with current intensities ranging from 90 to 160 μA . As the target depth of 4.2 mm was approached, we observed large field potentials that were time locked with the stimulus. Injections were targeted to sites at which follicle stimulation resulted in the largest amplitude responses. A 0.5% solution of CTB in 0.1 M NaPO_4 , pH 7.5, was iontophoretically injected at these sites, using the following parameters: 10 μA , alternating current, positive polarity, for 15 minutes.

The contralateral wMCx was then exposed in six of these rats. Preliminary experiments as well as previous studies (Donoghue and Wise, 1982; Weiss and Keller, 1994) used intracortical microstimulation to define the region of the wMCx. Low-intensity stimulation of the region 1.0–2.0 mm lateral and 1.0–3.5 mm rostral to Bregma evoked whisker movements. A pipette with a 10–20 μm tip diameter containing a 10% solution of biotinylated dextran amine (BDA; 10,000 MW; Molecular Probes, Eugene, OR) in 0.1 M phosphate buffer, pH 7.4, was advanced perpendicularly to the pial surface. The tracer was iontophoresed into three sites along the rostrocaudal axis of the contralateral wMCx, at depths ranging from 0.75 to 1.5 mm from the pial surface. Neighboring injections were approximately 0.5–1.0 mm apart, and injection parameters at each site were as follows: 7 μA , alternating current, positive polarity, for 5 minutes at each of three sites.

Seven to fourteen days after these surgical procedures, the rats were anesthetized with sodium pentobarbital (50 mg/kg, IP) and perfused transcardially with cold 0.9% saline for 10 minutes, followed by a phosphate-buffered 4% paraformaldehyde solution for 20 minutes. Brains were postfixed in the same fixative solution for 30 minutes at room temperature and then transferred to a 20% sucrose phosphate buffer solution and stored at 4°C for 24–48 hours.

Histology

Coronal or sagittal sections (35 μm thick) through the mid- and hindbrain were cut with a cryostat. To reveal BDA-labeled cortical afferents, sections were incubated in phosphate-buffered saline (PBS; pH 7.4) with 0.3% Triton X-100 for 30 minutes and then incubated in avidin-biotin complex (ABC Elite kit; 1:500; Vector Laboratories, Burlingame, CA) for 1 hour. The sections were washed in PBS, and the reaction product was visualized using nickel (NiSO_4)- and cobalt (CoCl_2)-enhanced diaminobenzidine (DAB; 0.1 mg/ml) and H_2O_2 (0.015%) in Tris in buffer, pH 7.2. To reveal somata retrogradely labeled with CTB, the same sections were incubated at room temperature in the following solutions: 1) 30 minutes in 5% normal donkey serum (Jackson ImmunoResearch Laboratories, West Grove, PA) in PBS, 2) overnight in a 1:15,000 dilution of a polyclonal anticholeragenoid antiserum raised in goat (List Biotechnology Laboratories, Campbell, CA), 3) several rinses in PBS, 4) 1 hour in biotinylated goat IgG raised in donkey (1:400; Jackson ImmunoResearch Laboratories), 5) several rinses in PBS, 6) 1 hour in ABC (1:500), and 7) 10–15 minutes in DAB and H_2O_2 in Tris buffer, pH 7.2. All sections were mounted, dehydrated in graded alcohols, cleared in xylene, and coverslipped.

Analysis

The distributions of retrogradely labeled neurons and labeled cortical afferent fiber terminals were plotted using NeuroLeucida (MicroBrightField, Colchester, VT) and transferred to rat brain maps (Paxinos and Watson, 1998). Each map reflects labeled neurons and terminals in two or three sections (coronal maps, Fig. 4) or in four to six sections (sagittal maps, Fig. 5)

from a single animal. We analyzed every other 35- μ m-thick section and plotted the location of every observed CTB-labeled cell and small clusters of BDA-labeled terminals. Therefore, the data shown in these plots are likely to underestimate the actual number of neurons projecting to wFMNs and of cortical afferent terminals. We accounted for differences between atlas maps and actual sections by using the background histo-logical staining to identify cytoarchitectonic features of nuclei, axons, and cells. The relative density of anterogradely and retrogradely labeled structures in different nuclei was assessed qualitatively by assigning density values ranging from 0 (no labeling) to 4 (most dense labeling; see below and Table 1). High-resolution digital photomicrographs were obtained with a PowerPhase (PhaseOne, Denmark) charge-coupled device (CCD) and stored on a Power Macintosh computer. All digitized images were analyzed on a G4 Macintosh computer using Photoshop (Adobe, Mountain View, CA). Image manipulations were restricted to linear level adjustments and image cropping.

RESULTS

We injected the retrograde tracer CTB to label physiologically identified wFMNs. The injection sites were discrete, ranging in diameter between 100 and 200 μ m, and were restricted to the lateral portion of the facial nucleus (Fig. 1). This finding is in agreement with reports that wFMNs—identified by retrograde labeling from the whisker follicles and from transected nerves that abolished whisker movements—are located almost exclusively in the lateral facial nucleus (Klein and Rhoades, 1985; Semba and Egger, 1986). Retrogradely labeled neurons were identified by their content of a brown reaction product, which filled the somata and dendrites (Fig. 2A). We identified labeled axons within the facial nerve, but CTB-labeled axons were not observed in any other brain structure. This finding is in agreement with reports that facial motoneurons project only to the facial nerve and have no recurrent axon collaterals (Fanardjian et al., 1983). Thus, all CTB labeling can be attributed to retrograde transport of the tracer. Furthermore, we observed tracer deposits exclusively in the facial nucleus and not in any structures traversed by the CTB-filled pipettes. For six of these rats, we made additional injections of the anterograde tracer BDA into the contralateral wMCx. The injections ranged in diameter between 75 and 125 μ m and were confined to cortical layers III–V. None of these injections encroached on the underlying white matter, suggesting that all labeled efferents originated from somata located in the wMCx (Fig. 1). We identified, in all midbrain and brainstem structures examined, BDA labeling exclusively in axons and their varicosities. This finding, and the black reaction product in these axons, allowed us to distinguish between BDA-labeled axons and CTB-labeled (brown) somata and dendrites.

Labeling in brainstem structures

Injections of CTB and BDA revealed multiple regions in the brainstem, metencephalon, and midbrain containing both retrogradely labeled cells and anterogradely labeled cortical afferents. Dense retrograde and anterograde labeling was seen bilaterally in most regions of the brainstem reticular formation, including the parvocellular (PCRt), gigantocellular (Gi), intermediate (IRt), and medullary (dorsal and ventral; MdD, MdV) subdivisions (Table 1). Examples of retrogradely labeled cells in the PCRt and MdD are illustrated in Figure 2A,B and the distribution of CTB-labeled neurons in brainstem structures is plotted in Figures 4 and 5. CTB-labeled neurons were broadly distributed throughout the reticular formation, but labeling was most dense in the more caudal regions. The cell bodies of the labeled reticular formation neurons ranged in size between 10 and 25 μ m and typically had two to four primary dendrites. The axons of these neurons were not observable. BDA-labeled cortical afferent terminals were also present bilaterally throughout the reticular formation. Ipsilaterally, terminals were present along the rostrocaudal extent of the reticular formation. Contralaterally, terminals were more dense in the rostral regions, such as the PCRt, Gi, and IRt, and extended into the MdD and, to a lesser extent, into the MdV. Labeled afferent terminals often closely apposed CTB-labeled

neurons and appeared to form presumptive synaptic contacts on their somata and proximal dendrites (Fig. 3A,B). Although labeling was prominent in several nearby brainstem regions, no labeled afferents were detected in the injected or the contralateral facial nucleus, nor was retrograde labeling detected in the contralateral facial nucleus.

Two additional nuclei of the caudal brainstem both projected to wFMNs and received inputs from the wMCx: the ambiguus nucleus, which contained dense labeling, and the prepositus nucleus, which contained more sparse labeling. In both cases, this CTB and BDA labeling appeared bilaterally but was most dense ipsilateral to the facial nucleus injection site. Dense ipsilateral projections to wFMNs were also found from the dorsal motor nucleus of the vagus (Fig. 2C), and sparse projections were observed from the caudal nucleus of the solitary tract. No BDA-labeled afferent fibers were observed in either of these nuclei. Previous reports document extensive projections from vestibular nuclei to the motoneurons located in the dorsolateral facial nucleus (Isokawa-Akesson and Komisaruk, 1987). However, our injections of CTB into the physiologically identified wFMNs in the lateral facial nucleus revealed only sparse retrogradely labeled neurons in these nuclei.

In the sensory trigeminal nuclei, CTB labeling was found almost exclusively ipsilaterally to the injection site. Sparse retrograde labeling was present in the principal trigeminal nucleus and in all three spinal trigeminal nuclei (oralis, interpolaris, caudalis) but was most extensive in the nucleus caudalis (Fig. 5, Table 1). We infrequently observed labeled cortical afferents in the nucleus oralis and interpolaris. These findings are consistent with previous reports of the connectivity between the spinal trigeminal nuclei and the facial nucleus (Erzurumlu and Killackey, 1979; Pinganaud et al., 1999) and of the afferent projections from the whisker motor cortex (Miyashita et al., 1994).

Labeling in the metencephalon

Retrogradely labeled cells in the metencephalon were located bilaterally, whereas BDA-labeled afferents were found predominantly contralaterally to the facial nucleus injection site (Fig. 4). Consistently with reports by Isokawa-Akesson and Komisaruk (1987), the lateral and medial parabrachial nuclei, as well as the Kölliker-Fuse nucleus, project extensively to wFMNs in both hemispheres. Previous studies in which anterograde tracers were injected into each of these nuclei confirm that they project to the lateral facial nucleus (Saper and Loewy, 1980; Korte et al., 1992), suggesting that our results were not affected by inadvertent labeling of axons of passage. The parabrachial and Kölliker-Fuse nuclei contained only sparse BDA-labeled fibers.

Extensive projections to wFMNs were identified from the paralemniscal, pedunculo-pontine tegmental (PPTg), and intertrigeminal nuclei, predominantly ipsilaterally to the facial nucleus injection site. Cortical afferent terminals in all of these nuclei were essentially absent. Consistent with our findings, a projection from the intertrigeminal nucleus to orofacial motor nuclei was previously reported by Travers and Norgren (1983). An earlier study using retrograde tracers showed a dense projection from the ventral nucleus of the lateral lemniscus to the lateral facial nucleus (Isokawa-Akesson and Komisaruk, 1987). By contrast, we find dense retrograde labeling just medially to this nucleus in the paralemniscal nucleus and in the PPTg. We attribute this difference to our more localized and discrete injection into identified wFMNs. Supporting this finding, Grofova and Keane (1991) reported a sparse, yet consistent, projection from the PPTg to the lateral facial motor neurons by using anterograde tract tracing. Combined with our present data, these findings clearly demonstrate that the PPTg is an important source of input to wFMNs.

Sparse to moderate anterograde and retrograde labeling was also observed bilaterally in the pontine reticular nucleus, in the subpretectal tegmental nucleus, and in the subcoeruleus.

nuclei. Components of the motor trigeminal complex, including the motor trigeminal and supratrigeminal nuclei, contained no, or only sparse, labeled neurons and terminals. Significantly, an anterograde tract-tracing study by Rokx et al. (1986) demonstrated that the supratrigeminal nucleus projects to intermediate and dorsal regions of the facial nucleus and only rarely to the lateral facial nucleus. Thus, the scarcity of retrograde labeling in the supratrigeminal nucleus in our study indicates that our injections were indeed confined to the lateral subdivision of the facial nucleus.

Labeling in the midbrain

We observed in the superior colliculus CTB-labeled neurons at the level of the red nucleus, primarily contralaterally to the facial nucleus injection site. Dense retrograde labeling was predominantly localized to the deep gray and intermediate white layers (Fig. 4) and extended into the deep white layers. BDA-labeled afferent fibers terminated predominantly in these same layers of the superior colliculus, where labeled terminals often closely apposed cell bodies labeled with CTB (Fig. 3D). These labeling patterns are consistent with the anterograde labeling data of Miyashita and Mori (1995), demonstrating that the superior colliculus is an important relay structure between the motor cortex and the facial nucleus. In addition, the deep mesencephalic nucleus received numerous cortical afferent terminals and projected heavily to wFMNs.

Retrograde and anterograde labeling in the red nucleus was entirely contralateral to the facial nucleus injection site (Fig. 4). In agreement with Daniel et al. (1987), we found CTB-labeled neurons throughout the rostrocaudal extent of the red nucleus, nearly exclusively in its parvocellular subdivision. Labeled cortical terminals were sparsely distributed throughout the nucleus. Although both anterograde labeling and retrograde labeling were often localized to the same region of the red nucleus, there was little overlap in their distribution fields, suggesting that cortical efferents provide little direct input to red nucleus neurons that project to wFMNs (Fig. 3E). Previous studies demonstrating afferent terminals from the red nucleus in the facial nucleus (Godefroy et al., 1998) are consistent with our finding and suggest that the dense retrograde labeling in the parvocellular red nucleus was not a consequence of labeling by fibers of passage. Another source of inputs from the red nucleus is the rubrospinal neurons, which branch and send axon collaterals to the facial nucleus, as shown by antidromic stimulation (Sarkissian and Fanardjian, 1984) and by double labeling in the red nucleus following injections of retrograde tracers into the facial nucleus and the spinal cord (Martin et al., 1983). In our study, the regions immediately surrounding the red nucleus also contained considerable amounts of both retrograde and anterograde labeling. These include the area just dorsal to the red nucleus as well as the parabrachial nucleus and the ventral tegmental relay zone.

Several nuclei of the central gray terminate on wFMNs. Retrograde labeling was observed through much of the rostrocaudal extent of the periaqueductal gray (PAG), mainly at the level of the oculomotor nuclei. Here, most labeling was confined to the lateral PAG, and sparse retrograde labeling was also observed in the dorsomedial PAG. Cortical afferents terminated in the lateral PAG, where they were found closely apposed to retrogradely labeled cells (Fig. 3C). Several nuclei involved in oculomotor control were found to project to the lateral facial nucleus, including the interstitial nucleus of the medial longitudinal fasciculus (mlf), parvocellular subdivision of the oculomotor nucleus, supraoculomotor PAG, Edinger-Westphal nucleus, and Darkschewitsch nucleus. There was a substantial projection from the interstitial nucleus of the mlf and the parvocellular subdivision of the oculomotor nucleus to wFMNs. The interstitial nucleus of the mlf also received numerous projections from the cortex, whereas the other oculomotor nuclei mentioned above received only sparse projections from the wMCx. Previous anterograde tract-tracing studies revealed a direct projection from the Edinger-Westphal nucleus to the lateral facial nucleus (Klooster et al., 1993), and orthodromic

stimulation of the PAG, interstitial nucleus of the mlf, and Darkschewitsch nucleus evokes a monosynaptic excitatory postsynaptic potential (EPSP) in facial motoneurons (Fanardjian and Manvelyan, 1987). It is important that we did not observe labeling in the oculomotor nucleus, a region shown previously to project to orbicularis oculi motoneurons located in the dorsal subdivision of the facial nucleus (Takada et al., 1984). The lack of labeling in this region supports the conclusion that our injections did not inadvertently label adjacent motoneuron pools.

DISCUSSION

The goal of this study was to determine which structures receive direct inputs from the wMCx and provide inputs to wFMNs, by combining retrograde and anterograde tract-tracing techniques. These analyses established the anatomical substrates for cortical influences on pre-FMNs and on whisking activity. In addition to the combination of retrograde and anterograde tract tracing in the same animal, two other important features distinguish this study from previous reports of afferent projections to wFMNs. First, we physiologically identified wFMNs located within the lateral facial nucleus by recording field potentials while electrically stimulating the whisker follicles. Subsequent analysis revealed that all injection sites were localized exclusively to the lateral facial nucleus. As a result, we were able to demonstrate that certain premotoneurons that project to other divisions of the facial nucleus rarely target the wFMNs (e.g., oculomotor nucleus, supratrigeminal nucleus). Second, the retrograde tracer CTB used in this study has been reported to be twice as sensitive as tracers used in earlier reports (Horikawa and Powell, 1986). As a result, in comparison with previous reports, the number and density of retrogradely labeled cells were dramatically increased, allowing us to describe more accurately the projections to the wFMNs. Furthermore, we identified several nuclei previously not known to project to wFMNs as well as several nuclei that provide bilateral inputs to the wFMNs.

Our data indicate that wFMNs receive projections from several brainstem, metencephalic, and midbrain structures. In addition, many of the structures that project to wFMNs also receive direct inputs from the wMCx, suggesting that these regions are important for cortical regulation of whisker movements. Regions where retrograde and anterograde distributions overlap most extensively include the brainstem PCRt, Gi, IRt, MdD, MdV, ambiguus nucleus, and midbrain superior colliculus and deep mesencephalic nucleus. Other nuclei that contain less dense regions of combined anterograde and retrograde labeling include the interstitial nucleus of mlf, the pontine reticular formation, and the lateral PAG.

Pre-wFMNs that also receive dense inputs from the wMCx are likely to be a key component in a CPG involved in the regulation and modulation of rhythmic whisking activity. Supporting evidence for a whisking CPG is derived from a recent study demonstrating that sensory deafferentation does not affect the kinematics of exploratory whisking (Gao et al., 2001a). In addition, whole-cell current-clamp recordings from identified wFMNs in vitro reveal that these neurons fire spontaneously at 5–10 Hz and that this repetitive firing requires activation of glutamate receptors (Hattox et al., 2000). This suggests that the postulated CPG is present in the isolated brainstem slice preparation and that rhythmic firing of wFMNs is not an intrinsic property of these neurons but instead requires synaptic inputs from pre-FMNs. The present data indicate that several structures in the mid- and hindbrain may be involved in controlling whisker movements. In the following sections we discuss the potential role of pre-wFMNs in generating *rhythmic* whisking.

Brainstem reticular formation

We found that pre-FMNs in the PCRt, Gi, IRt, MdD, and MdV provide dense bilateral projections to wFMNs. Many of these labeled pre-FMNs were closely apposed to anterogradely

labeled cortical afferents, where presumptive synaptic contacts were made. Several lines of anatomical and physiological evidence support the conclusion that pre-FMN in the brainstem reticular formation are part of a CPG that controls rhythmic whisking. Neurons in this region are involved in other types of rhythmic motor acts in mammals, such as licking, mastication, and locomotion (Drew et al., 1986; Nozaki et al., 1993; Chen et al., 2001; for review see Buttner-Ennever and Holstege, 1986). Similarly, the reticular formation in birds contains the CPG for rhythmic acts such as pecking and jaw movements (Berkhoudt et al., 1982; Wild et al., 1985). Micro-stimulation of neurons in the PCRT evokes rhythmic whisking (Isokawa-Akesson and Komisaruk, 1987), suggesting that these neurons may control whisking behaviors by driving wFMNs. Finally, electrical stimulation of neurons in the reticular formation evokes monosynaptic EPSPs in facial motoneurons in the cat (Fanardjian and Manvelyan, 1987). All of these findings suggest that neurons in the brainstem reticular formation may be a part of the whisking CPG and that they are important for relaying the modulatory influences of the wMCx.

Trigeminal nuclei

The trigeminal nerve carries sensory inputs from the whiskers and innervates the principal trigeminal nucleus as well as the three spinal trigeminal nuclei (oralis, interpolaris, and caudalis). Retrograde labeling in the spinal trigeminal nuclei was sparse, exclusively ipsilateral, and observed predominantly in the nucleus caudalis. Labeled afferent fibers were essentially absent from all of the sensory trigeminal nuclei. Stimulation in the dorsal spinal trigeminal nucleus can evoke whisker movements (Isokawa-Akesson and Komisaruk, 1987). Thus, the trigeminal nuclei may be part of a closed-loop system that exerts feedback control on whisking. However, recent findings indicate that rhythmic whisking is unaffected by lesions of the trigeminal nerve (Gao et al., 2001a). This indicates that rhythmic whisking is not dependent on sensory feedback but instead depends on a subcortical CPG. In the motor trigeminal nuclei, there was only minimal overlap between retrograde and anterograde labeling. This suggests that the motor trigeminal complex, a principal component in rhythmic jaw movements, is not involved in directly generating or controlling whisker movements.

Red nucleus

Retrogradely labeled pre-FMN were located exclusively in the contralateral red nucleus, primarily in its dorsal regions. Labeled afferent fibers were also distributed in this region of the nucleus, however, labeled somata and fibers were rarely observed closely apposed to one another. It is possible that the red nucleus is involved in relaying inputs from the olivocerebellar system to wFMNs (Welsh et al., 1995). This is of interest because the characteristic frequency of rhythmic activity in the olivocerebellar system is similar to the frequency of rhythmic whisking (see, e.g., Lang et al., 1997). However, inactivation of the inferior olive does not affect exploratory whisker movements (Semba and Komisaruk, 1984), so this rhythmic activity may not be causally related to these movements. Furthermore, stimulation of the red nucleus does not reliably evoke whisker movements (Isokawa-Akesson and Komisaruk, 1987). Thus, the role of the red nucleus in modulating whisker movements is at present unclear.

Paralemniscal nucleus and the PPTg

These two nuclei project heavily to wFMNs but receive few, if any, inputs from the wMCx. The paralemniscal nucleus has been most closely associated with movement of the pinna muscles (Henkel and Edwards, 1978) but more recently has been shown to project to auricular motoneurons (Panneton and Martin, 1983) and implicated in the production of two repetitive movements, inspiration (Gaytan and Pasaro, 1998) and suckling (Li et al., 1999). The PPTg is associated with motor functions, arousal, and sleep, and lesions of this nucleus cause Parkinsonian-like motor disturbances (Hirsch et al., 1987).

Respiratory and cardiac regulation

Whisker movements and respiration are coordinated during exploratory sniffing (Welker, 1964), and inputs from nuclei involved in regulation of the respiratory and circulatory systems may be involved in this coordinated activity. The parabrachial, Kölliker-Fuse, and intertrigeminal nuclei, as well as the dorsal motor nucleus of the vagus, have all been associated with the regulation of breathing and heart rate (Chamberlin and Saper, 1998). In addition, the intertrigeminal nucleus has been implicated in the generation of rhythmic jaw movements (Zhang and Sasamoto, 1990). Our studies show that these four nuclei project heavily to the ipsilateral wFMNs. However, cortical afferents were very sparse in these regions, so these nuclei are unlikely to mediate cortical regulation of wFMNs. In contrast, the ambiguous nucleus and the lateral PAG, also involved in the regulation of breathing and heart rate, both contain moderate to dense retrograde and anterograde labeling. Projections from the ambiguous nucleus are of particular interest, in that the ambiguous nucleus has been proposed as a CPG for swallowing (Broussard and Altschuler, 2000).

Visuomotor nuclei

Our results show dense retrograde labeling in the superior colliculus contralateral to the facial nucleus injection site and show that this region also receives dense projections from the wMCx. Electrical stimulation of the superior colliculus elicits contralateral whisker movements (McHaffie and Stein, 1982). The superior colliculus also forms reciprocal connections with trigeminal nuclei relaying vibrissal information (Stein et al., 1975; Isokawa-Akesson and Komisaruk, 1987) and is likely to be involved in integrating inputs from motor behaviors with inputs from somatosensory, visual, and auditory sensory modalities.

In addition to the superior colliculus, several nuclei involved in oculomotor behaviors project bilaterally to the wFMNs and receive inputs from the wMCx. These include interstitial nucleus of the mlf; parvocellular subdivision of the oculomotor nucleus; supraoculomotor PAG; and Edinger-Westphal, Darkschewitsch, and prepositus nuclei. These nuclei are all associated with visuomotor behaviors and have been shown to project to areas involved in somatosensory and motor control. Stimulation of many of these regions has also been shown to evoke monosynaptic EPSPs in FMNs (Fanardjian and Manvelyan, 1987).

In addition to wFMNs, the facial nucleus contains motoneurons that control ocular muscles, raising the possibility that the tracer inadvertently labeled neurons that project to motoneuron pools adjacent to wFMNs. There are several reasons why we believe that this is not the case. First, as discussed above, our discrete injection sites were localized to physiologically identified wFMNs, were restricted to the lateral facial nucleus, and in almost every case were confined to the ventral region of the lateral facial nucleus. By contrast, motoneurons innervating the orbicularis oculi muscles are found in the dorsal and intermediate subdivisions of the facial nucleus (Watson et al., 1982). Second, whereas orbicularis oculi motoneurons were shown to receive inputs from premotoneurons located in the oculomotor nucleus (Takada et al., 1984), we found no retrograde labeling in the oculomotor nucleus, suggesting that our injections did not inadvertently label oculomotor motoneuron pools. Thus, our finding of widely distributed wFMN-projecting neurons in regions associated with eye movements suggests that these structures are involved in the coordination of head, eye, and whisker movements that occur during exploratory behaviors (Welker, 1964).

The integral contribution of rodents' whisker movements in exploring their sensory environment is made apparent by the number of structures providing inputs to wFMNs involved in processing sensory information. Although several structures may relay wMCx outputs to wFMNs, the preponderance of evidence suggests that the brainstem reticular formation is a key structure in generating whisker movements. In addition to being a site of extensive overlap

in the distributions of retrograde and anterograde tracer labeling, reticular formation neurons have been shown to be involved in the generation of other rhythmic movements. Several additional structures of the midbrain and metencephalon also contain overlapping distributions of the two tracers. It is possible that these structures are important in integrating whisker movements with a wide range of sensory inputs. Therefore, although cortical control of whisking undoubtedly involves interactions among several neuronal circuits, our findings suggest that pre-FMNs in the brainstem reticular formation are the final common pathway for cortical and subcortical regulation of rhythmic whisking.

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Abbreviations

3, oculomotor nucleus
 3PC, oculomotor nucleus, parvocellular subdivision
 A5, A5 noradrenaline cells
 7, facial nucleus
 7n, facial nerve
 10, dorsal motor nucleus of the vagus
 Amb, ambiguus nucleus
 APT, anterior pretectal nucleus
 C1, C1 adrenaline cells
 csc, commissure of superior colliculus
 Dk, nucleus of Darkschewitsch
 DLL, dorsal nucleus of lateral lemniscus
 DLPAG, dorsolateral periaqueductal gray
 DMPAG, dorsomedial periaqueductal gray
 DMSp5, dorsomedial spinal trigeminal nucleus
 DMTg, dorsomedial tegmental area
 DpG, deep gray layer of the superior colliculus
 DpGi, dorsal paragigantocellular nucleus
 DpMe, deep mesencephalic nucleus
 DpWh, deep white layer of the superior colliculus
 ELm, epilemniscal nucleus
 EW, Edinger-Westphal nucleus
 Gi, gigantocellular reticular nucleus
 GiA, gigantocellular reticular nucleus, alpha
 GiV, gigantocellular reticular nucleus, ventral
 I3, interoculomotor nucleus
 I5, intertrigeminal nucleus
 IMLF, interstitial nucleus of the medial longitudinal fasciculus
 InG, intermediate gray layer of the superior colliculus
 InWh, intermediate white layer of the superior colliculus
 IO, inferior olive
 IRt, intermediate reticular nucleus
 KF, Kölliker-Fuse nucleus
 ll, lateral lemniscus
 LPAG, lateral periaqueductal gray
 LPB, lateral parabrachial nucleus
 LPGi, lateral perigigantocellular nucleus
 m5, motor root of the trigeminal nerve

MA3, medial accessory oculomotor nucleus
 MdD, medullary reticular formation, dorsal
 MdV, medullary reticular formation, ventral
 Me5, mesencephalic trigeminal nucleus
 Min, minimus nucleus
 ml, medial lemniscus
 mlf, medial longitudinal fasciculus
 Mo5, motor trigeminal nucleus
 MPB, medial parabrachial nucleus
 MVe, medial vestibular nucleus
 P5, peritrigeminal zone
 P7, perifacial zone
 PaR, pararubral nucleus
 PBP, parabrachial pigmented nucleus
 PC5, parvocellular motor trigeminal nucleus
 PCRt, parvocellular reticular nucleus
 PCRtA, PCRt, alpha part
 PL, paralemniscal nucleus
 PnC, pontine reticular nucleus, caudal part
 PnO, pontine reticular nucleus, oral part
 PoT, posterior thalamic nucleus, triangular
 PPTg, pedunculopontine tegmental nucleus
 Pr, prepositus nucleus
 Pr5, principal sensory trigeminal nucleus
 py, pyramidal tract
 RMC, red nucleus, magnocellular part
 RPC, red nucleus, parvocellular part
 RPO, rostral periolivary region
 RR, retrorubral nucleus
 RRF, retrorubral field
 rs, rubrospinal tract
 RtTg, reticulotegmental nucleus of the pons
 RVL, rostroventrolateral reticular nucleus
 s5, sensory root of trigeminal nerve
 scp, superior cerebellar peduncle
 SG, supragenulate nucleus
 SNR, substantia nigra, reticular part
 sol, solitary tract
 Sol, nucleus of the solitary tract
 Sp5, spinal trigeminal tract
 Sp5C, spinal trigeminal nucleus, caudal part
 Sp5I, spinal trigeminal nucleus, interpolar part
 Sp5O, spinal trigeminal nucleus, oral part
 SPTg, subpeduncular tegmental nucleus
 SpVe, spinal vestibular nucleus
 Su3, supraoculomotor periaqueductal gray
 Su5, supratrigeminal nucleus
 SubCA, subcoeruleus nucleus, alpha part
 SubCD, subcoeruleus nucleus, dorsal part
 SubCV, subcoeruleus nucleus, ventral part
 ts, tectospinal tract
 VLL, ventral nucleus of the lateral lemniscus
 VLTg, ventrolateral tegmental area

vsc, ventral spinocerebellar tract
 VTRZ, visual tegmental relay zone

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Fig. 1. Photomicrographs of retrograde and anterograde tracer injection sites. **A:** CTB injection site in the physiologically identified lateral facial nucleus. A drawing of the corresponding coronal section, with the facial nucleus (7) highlighted, is shown in the **inset**. **B:** BDA injection sites in the wMCx. Boundaries between cortical layers occur approximately halfway between Roman numerals. Scale bar = 400 μ m.

Fig. 2. Photomicrographs of retrogradely labeled cells in the brainstem. Examples of CTB-labeled neurons in the PCRt (**A**), the MdD (**B**), and the dorsal motor nucleus of the vagus (**C**). Scale bars = 20 μ m in A,C, 50 μ m in B.

Fig. 3. Photomicrographs of BDA-labeled cortical afferents (black) abutting retrogradely labeled pre-wFMNs (brown). **A:** IRT. **B:** PCRt; B is a montage of images at two focal planes. **C:** PAG. **D:** Superior colliculus. **E:** An example of infrequent apposition between cortical afferents and labeled pre-wFMNs in the red nucleus. Scale bars = 10 μ in A–C, 20 μ in D,E.

Fig. 4. Distribution of retrogradely labeled neuronal cell bodies and anterogradely labeled cortical afferent fiber terminals in the brainstem and midbrain plotted on a series of coronal map sections. Each labeled neuron is represented by a red circle, and small clusters (three to five) of labeled terminals are represented by a green triangle. Each map represents labeled neurons and terminals in two or three sections from one animal. Maps are arranged from left to right in series beginning with the most caudal section; the anterior-posterior level of each section, relative to Bregma, is indicated below each map. The facial nucleus injection site was in the left hemisphere. Maps modified, with permission, from Paxinos and Watson (1998). Scale bar = 1 mm.

Fig. 5. Distribution of retrogradely labeled neuronal cell bodies in brainstem structures following injection of CTB in the physiologically identified lateral facial nucleus. Each labeled neuron is represented by a solid circle plotted on a series of sagittal map sections. Each map represents labeled neurons in five to seven sections from one animal; the medial-lateral level of each section, relative to the midline, is indicated to the left of each map. Sagittal map sections shown are ipsilateral to the facial nucleus injection site. Maps modified, with permission, from Paxinos and Watson (1998). A low-magnification sagittal section corresponding to lateral = 1.9 mm is shown at top right. Scale bar = 1 mm.

TABLE 1

Relative Density of BDA-Labeled Fibers and CTB-Labeled Neurons in Various Structures¹

<i>Ipsilateral</i>			<i>Contralateral</i>	
BDA	CTB		BDA	CTB
Brainstem				
+++	+++	Parvocellular reticular formation	++	++
++++	+++	Gigantocellular reticular formation	+++	++
+++	+++	Intermediate reticular formation	++	++
+++	+++	Medullary reticular formation, dorsal	++	++
++	+++	Medullary reticular formation, ventral	+	++
+++	+++	Ambiguous nucleus	++	++
	+++	Dorsal motor nucleus of the vagus		
	+	Nucleus oralis		
	+	Nucleus interpolaris		
	++	Nucleus caudalis		
	++	Nucleus of the solitary tract		+
++	+	Vestibular nuclei	++	
Metencephalon				
+	++++	Kölliker-Fuse nucleus	+	++
	++	Lateral Parabrachial nucleus	+	+
	++	Medial Parabrachial nucleus		++
	++++	Paralemniscal nucleus	+	++
	+++	Pedunculopontine tegmental nucleus		++
+	++	Subpeduncular tegmental nucleus	++	+
+	+++	Intertrigeminal nucleus		+
+	++	Pontine reticular nucleus	++	++
+	++	Subcoeruleus	+	+
	+	Principal trigeminal nucleus		
Midbrain				
+	+	Superior colliculus (DpG/InWh)	+++	+++
++	+++	Deep mesencephalic nucleus	+++	+++
		Red nucleus, parvocellular part	+	++++
		Red nucleus, magnocellular part	+	+
+	+	Parabrachial nucleus	++	+
+	++	Periaqueductal gray, lateral	++	+
+	+	Periaqueductal gray, dorsomedial	+	+
++	++	Interstitial nucleus of mlf	+++	++
+	++	Edinger-Westphal nucleus	+	++
+	++	Oculomotor nucleus, parvocellular	+	++++
+	++	Supraoculomotor periaqueductal gray	++	+
+	+	Darkschewitsch nucleus	+	
+	+	Substantia nigra, reticular part	+	

¹The density of anterogradely labeled (BDA) cortical affrents and retrogradely labeled (CTB) somata is expressed according to the following scale: +, sparse; ++, moderate; +++, dense; +++, very dense. *Ipsilateral* and *contralateral* refer to the facial nucleus injection site.