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AFFERENT SUBCORTICAL CONNECTIONS INTO THE MOTOR CORTICAL LARYNX AREA IN THE RHESUS MONKEY

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Abstract—In three rhesus monkeys (*Macaca mulatta*), the inferior motor cortex was explored by electrical stimulation for sites yielding vocal fold adduction. The retrograde tracer wheat germ-agglutinin-conjugated horseradish peroxidase was injected into the effective sites. Within the forebrain, retrogradely labeled cells were found in the claustrum, basal nucleus of Meynert, substantia innominata, extended amygdala, lateral and posterior hypothalamic area, field H of Forel, and a number of thalamic nuclei with the strongest labeling in the nuclei ventralis lateralis, ventralis posteromedialis, including its parvocellular part, medialis dorsalis and centrum medianum, and weaker labeling in the nuclei ventralis anterior, ventralis posterolateralis, intermediodorsalis, paracentralis, parafascicularis and pulvinaris anterior. In the midbrain, labeling was found in the deep mesencephalic nucleus, ventral tegmental area, and substantia nigra. In the lower brainstem, labeled cells were found in the pontine reticular formation, median and dorsal raphe nuclei, medial parabrachial nucleus, and locus coeruleus. The findings are discussed in terms of the possible role of these structures in voluntary vocal control.

Key words: motor cortex, larynx area, vocal fold control, thalamus, phonation, neuroanatomy.

Voluntary control of phonation, such as in speech and song, requires the intactness of the lower precentral cortex, that is part of the motor cortex, which contains the oral and laryngeal muscle representation. If this region is destroyed bilaterally, pseudobulbar palsy emerges, a syndrome characterized by a complete loss of voluntary control over the oral and laryngeal muscles (Foix et al., 1926). If, in contrast, the lower precentral cortex is activated by localized electrical brain stimulation, movements of the lips, tongue and vocal folds are obtained (Foerster, 1936; Penfield and Bordley, 1937). If, during phonation, the neural activity is monitored using functional magnetic resonance imaging or positron emission tomography, phonation-correlated activity is found in the lower precentral cortex (Bookheimer et al., 2000; Perry et al., 1999). These findings indicate that the lower precentral gyrus is involved in phonation control. Not all kinds of phonation, however, depend upon this region. Destruction of the lower precentral cortex in the monkey does not impair vocal communication (Kirzinger and Jürgens, 1982). As monkey calls, in contrast to human speech and song, do not need to be learned, but can be produced by the animals without ever having heard them from conspecifics (Hammerschmidt et al., 2001), it may be concluded that the lower precentral cortex is crucial for the control of learned vocal patterns, but not of innate vocal patterns. From the fact that monkeys do have a motor cortical larynx representation, that is, an area producing isolated vocal fold movements when electrically stimulated (Hast and Milojevic, 1966; Hast et al., 1974), it may be concluded that monkeys have at least some voluntary control over vocal fold movements. This control possibly is non-vocal, being related, for instance, to abdominal straining movements carried out during large jumps, lifting heavy weights, defecation or giving birth. The presence of such a voluntary control justifies the use of non-human primates as models in the study of human voluntary vocal fold control.

In the present study, we investigated the subcortical input into the motor cortical larynx area of the rhesus monkey. This study supplements a recent study on the cortical input of the motor cortical larynx area in the same species (Simonyan and Jürgens, 2004). The subcortical afferent connections of the motor cortical larynx area have been studied until now in only one other species, namely the squirrel monkey (Jürgens, 1982). A comparison of the results from the rhesus monkey and squirrel monkey may help to identify anatomical connections of the primate motor cortical larynx area in general, that is, connections common to all primates, including man.

EXPERIMENTAL PROCEDURES

The experiments were carried out in three rhesus monkeys, *Macaca mulatta*, weighing 3.0–6.8 kg. The same animals were used as in a previous study on the cortical afferent connections of the motor cortical larynx area (Simonyan and Jürgens, 2004). The animals were captivity-bred in the German Primate Center (Göttingen, Germany) and kept in groups in large cages (>25 m^3) with an enriched environment. All experimental procedures were approved by the Animal Ethics Committee of the district government Braunschweig, Lower Saxony, Germany, and conformed to National Institutes of Health guidelines. Care was taken to minimize the number of animals used and their suffering.

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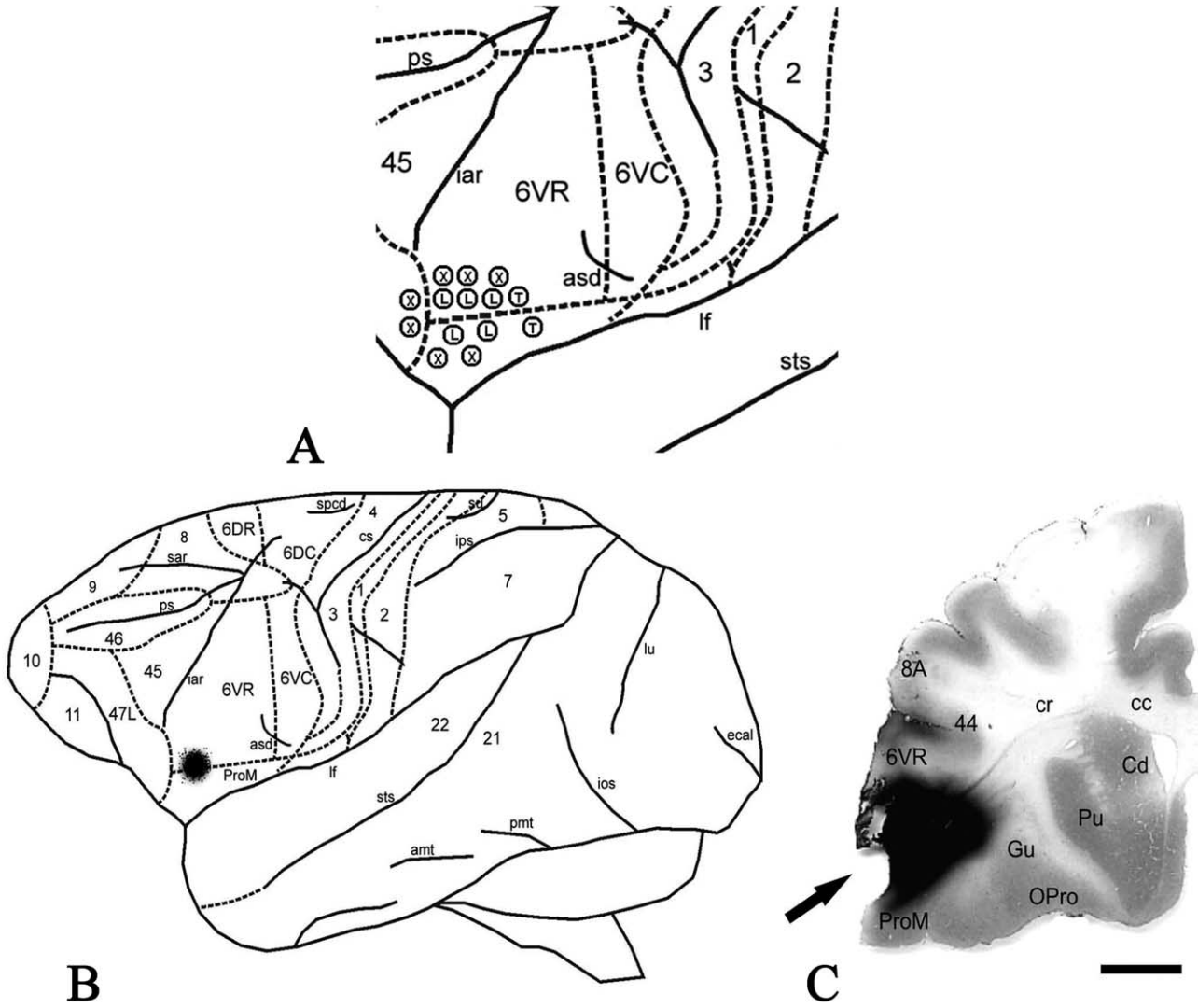

Fig. 1. (A) Lateral view of the left inferior frontal cortex of animal 1, showing the stimulation sites tested. L, glottis closure, T, tongue movement, X, no motor response. (B) Lateral view of a schematized rhesus monkey brain, showing WGA-HRP injection area common to all three animals (solid black area). (C) Low-power photograph of a frontal section of animal one at the level of maximum injection area (arrow). Cytoarchitectonic parcellation and nomenclature according to Paxinos et al. (2000). Scale bar=7 mm.

Surgery and injection

The monkeys were anesthetized with a mixture of 50 mg ketamine, 10 mg xylazine and 0.1 mg atropine sulfate in 1.0 ml sterile water, given intramuscularly. The injection was repeated every 30–45 min during the course of the operation. The monkeys were placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, USA). The skin in the left temporal region, together with the underlying temporal muscle and fascia were incised. Then the bone was removed above the inferior motor cortex in a diameter of 15–20 mm.

In all animals, electrical brain stimulation was carried out transdurally, using a monopolar stainless steel electrode (shaft diameter: 250 μm). All animals were explored on the left side for better comparability. The exploration region was limited by the inferior branch of the arcuate sulcus rostrally, the subcentral dimple caudally, the Sylvian fissure ventrally, and a line 12 mm above the Sylvian fissure dorsally (Fig. 1A). This region was found by Hast and colleagues (1974) to contain the cricothyroid and thyroarytenoid muscle representation in the rhesus monkey. The stimulus parameters used were monophasic rectangular pulses of 1 ms pulse width, 70 Hz repetition rate, 3 s train duration, and 500 μA maximum current (impedance: 14 kOhm). Thresholds for evoking vocal fold movements ranged between 320 and 410 μA. The type of vocal fold movement obtained was an isolated bilateral symmetrical adduction. Vocal folds were examined by means of indirect laryngoscopy.

Effective sites were injected with a retrograde tracer by the aid of a micromanipulator-driven 1.0 μl Hamilton microsyringe (cannula diameter of 0.47 mm). One microliter of 3% WGA-HRP (lectin from Triticum vulgaris conjugated to Sigma type VI horseradish peroxidase, Fa.; Sigma Chemical Co., St. Louis, USA) in isotonic sodium chloride solution was injected in animals 1 and 2. Animal 3 received two injections of 0.8 μl each, 1.0 mm apart from each other. After the injections, the bone defect was closed with titanium-reinforced Gore-Tex regenerative material (W.L. Gore & Associates, Inc., AZ, USA). The wound was closed by suturing the muscle fascia and skin in two layers. After the operation, the animals were returned to their home cage. They received supple-

Abbreviations used in the figures

AA	Anterior amygdaloid area
ac	Anterior commissure
acp	Anterior commissure, posterior part
AHA	Anterior hypothalamic area, anterior part
AHi	Amygdalohippocampal area
amt	Anterior middle temporal sulcus
APT	Anterior pretectal nucleus
APul	Anterior pulvinar
asd	Anterior subcentral dimple
Aq	Aqueduct
B	Basal nucleus (Meynert)
BIC	Nucleus of the brachium of the inferior colliculus
BL	Basolateral amygdaloid nucleus
BM	Basomedial amygdaloid nucleus
bsc	Brachium of the superior colliculus
BST	Bed nucleus of the stria terminalis
Cb1	Cerebellar lobe 1
Cb2	Cerebellar lobe 2
Cb3	Cerebellar lobe 3
cc	Corpus callosum
Cd	Caudate nucleus
Ce	Central amygdaloid nucleus
chp	Choroid plexus
CIC	Central nucleus of inferior colliculus
Cl	Claustrum
CL	Centrolateral thalamic nucleus
CM	Central medial thalamic nucleus
CMn	Centromedian thalamic nucleus
CnF	Cuneiform nucleus
Co	Cortical amygdaloid nucleus
cp	Cerebral peduncle
cr	Corona radiata
cs	Central sulcus
ctg	Central tegmental tract
DCIC	Dorsal cortex of the inferior colliculus
Dk	Nucleus of Darkschewitsch
DM	Dorsomedial hypothalamic nucleus
DMTg	Dorsomedial tegmental area
DTg	Dorsomedial tegmental nucleus
DpMe	Deep mesencephalic nucleus
DR	Dorsal raphe nucleus
EA	Extended amygdala
ec	External capsule
ecal	External calcarine sulcus
ECIC	External cortex of the inferior colliculus
eml	External medullary lamina
En	Endopiriform nucleus
ex	Extreme capsule
f	Fornix
fr	Fasciculus retroflexus
GPe	External globus pallidus
GPi	Internal globus pallidus
Gu	Gustatory cortex
Gus	Gustatory thalamic nucleus (VPMpc)
H1	Field of Forel
Hb	Habenula
Hip	Hippocampus
I5	Intertrigeminal nucleus
IAM	Interanteromedial thalamic nucleus
iar	Inferior arcuate sulcus
ic	Internal capsule
ICo	Intercollicular nucleus
IMD	Intermediodorsal thalamic nucleus
ios	Inferior occipital sulcus
IP	Interpeduncular nucleus
ips	Intraparietal sulcus
La	Lateral amygdaloid nucleus
LC	Locus coeruleus
LDS	Lateral dorsal thalamic nucleus
lf	Lateral fissure
LG	Lateral geniculate body
LH	Lateral hypothalamic area
Li	Linear nucleus of the raphe
Lim	Limitans thalamic nucleus
ll	Lateral lemniscus
LLd	Dorsal nucleus of lateral lemniscus
LLv	Ventral nucleus of lateral lemniscus
LPB	Lateral parabrachial nucleus
LPul	Lateral pulvinar
LSO	Lateral superior olive
LV	Lateral ventricle
lu	Lunate sulcus
MDC	Mediodorsal thalamic nucleus, caudal part
MDD	Mediodorsal thalamic nucleus, dorsal part
MDL	Mediodorsal thalamic nucleus, lateral part
MDM	Mediodorsal thalamic nucleus, medial part
ME	Median eminence
Me	Medial amygdaloid nucleus
MG	Medial geniculate body
ml	Medial lemniscus
mlf	Medial longitudinal fasciculus
MN	Mammillary nucleus
MnR	Median raphe nucleus
Mo5	Motor trigeminal nucleus
MPB	Medial parabrachial nucleus
MPul	Medial pulvinar
MSO	Medial superior olive
OPro	Orbital proisocortex
opt	Optic tract
ox	Optic chiasm
PAG	Periaqueductal gray
PaS	Parasubiculum
PC	Paracentral thalamic nucleus
PCom	Nucleus of the posterior commissure
PF	Parafascicular thalamic nucleus
PH	Posterior hypothalamic area
Pi	Pineal gland
pmt	Posterior middle temporal sulcus
Pn	Pontine gray
PN	Paranigral nucleus
PnC	Pontine reticular nucleus, caudal part
PnO	Pontine reticular nucleus, oral part
PnR	Pontine raphe nucleus
Po	Posterior thalamic nucleus
POA	Preoptic area
POl	Periolivary region
PP	Peripeduncular nucleus
PPTg	Pedunculopontine tegmental nucleus
Pr	Prepositus nucleus
Pr5	Principal sensory trigeminal nucleus
PRF	Prerubral field
ProM	Promotor cortex
ps	Principal sulcus
Pu	Putamen
Pul	Pulvinar
PVG	Paraventricular gray
py	Pyramidal tract
Re	Reticular nucleus
RMC	Red nucleus, magnocellular part
RMg	Raphe magnus nucleus
RPC	Red nucleus, parvicellular part
Rt	Reticular thalamic nucleus
RtTg	Reticulotegmental nucleus of the pons
s5	Sensory root of the trigeminal nerve
sar	Superior arcuate sulcus
SC	Superior colliculus
SI	Substantia innominata
sm	Stria medullaris of the thalamus
SN	Substantia nigra
SO	Supraoptic nucleus
spcd	superior precentral dimple
st	Stria terminalis
STh	Subthalamic nucleus
sts	Superior temporal sulcus
su	Superior postcentral dimple
SubC	Subcoeruleus nucleus
ts	Tectospinal tract
Tz	Nucleus of the trapezoid body
tz	Trapezoid body
VAL	Ventral anterior thalamic nucleus, lateral part
VAM	Ventral anterior thalamic nucleus, medial part
VAMC	Ventral anterior thalamic nucleus, magnocellular part
VLL	Ventral lateral thalamic nucleus, lateral part
VLM	Ventral lateral thalamic nucleus, medial part
VMH	Ventromedial hypothalamic nucleus
VP	Ventral pallidum
VPL	Ventral posterolateral thalamic nucleus
VPM	Ventral posteromedial thalamic nucleus
VTA	Ventral tegmental area
xscp	Decussation of the superior cerebellar peduncle
ZI	Zona incerta
1 . . . 47	Brodmann area 1 . . . 47
3n	Oculomotor nerve
3N	Oculomotor nucleus
3V	3rd ventricle
4V	4th ventricle
6N	Abducens nucleus
7n	Facial nerve

mental doses of an antibiotic (Baytril 0.1 ml/kg body weight) for the following days.

Fixation and histological processing

After a survival period of 3 days, the monkeys were deeply narcotized with an overdose of 16% pentobarbital sodium. Transcardial perfusion was performed with 2 l of 0.9% saline (37 °C) and 4 l of fixative solution (1% paraformaldehyde/1.25% glutaraldehyde in 0.1 M phosphate buffer of pH 7.4 at room temperature), followed by cold 10% and 20% sucrose buffers, 1 l each (4 °C, pH 7.4). The brains were removed from the skull and stored in 30% sucrose buffer for cryoprotection at 4 °C for 6–7 days. The brain blocks then were cut in the stereotaxic frontal plane at 45 μm on a freezing microtome (Frigocut, 2800; Reichert-Jung, Nussloch, Germany); every second section was taken. The sections were collected in 0.1 M phosphate buffer (pH 7.4) and stored at 4 °C.

Immunohistochemical tracer identification was carried out according to a modification of the procedure described by Mesulam (1978). Briefly, after several rinsings in distilled water, the sections were immersed in an incubation solution (mixture of 100 mg sodium nitroferricyanide, 5 ml sodium acetate buffer of pH 3.3, 92.5 ml distilled water with 5 mg tetramethylbenzidine in 2.5 ml absolute alcohol) for 20 min on a rocker table at room temperature. The following enzymatic reaction was initiated by adding 400 μl of 0.3% H_2O_2 to each 10 ml of incubation solution at room temperature. Enzymatic reaction time lasted another 20 min. After staining, the sections were rinsed five times in a sodium acetate buffer of pH 3.3 and were mounted on doubly gelatin-coated slides from cold gelatin solution. After drying overnight, half of the sections were counterstained with Cresyl Violet. All sections were dehydrated through an ascending alcohol series, cleared with xylene and coverslipped with DePeX.

Data analysis

The sections were evaluated microscopically (Olympus BX 50; Olympus Optical Co., Ltd., Japan) under bright and dark field illumination. We used the stereotaxic atlas of the rhesus monkey brain of Paxinos et al. (2000) to identify the labeled structures. Photo documentation was made with the help of a digital camera (Olympus C-3030) and proper software (Olympus-DpSoft, version 3.0 for Windows 98/2000, Olympus Optical Co., Ltd.). Brain diagrams were drawn on the basis of the Paxinos et al. (2000) stereotaxic atlas, and the labeled cells were directly plotted.

RESULTS

In all three animals, the injection sites were located about 5 mm above the Sylvian fissure between the inferior ramus of the arcuate sulcus rostrally and the subcentral dimple posteriorly, corresponding cytoarchitectonically to areas 6VR and ProM of Paxinos et al. (2000; Fig. 1B). The tracer injection involved all six cortical layers (Fig. 1C). In animal 3, the two injection sites merged into each other. Injection sites were characterized by an intensely labeled central core of homogeneous reaction product, surrounded by a broad halo.

As the anterograde subcortical projections have been reported already in an earlier study (Simonyan and Jürgens, 2003), we will describe here only the retrograde subcortical projections.

The number of retrogradely labeled subcortical areas ranged between 37 and 22, depending on the animal. Altogether, 30 cytoarchitectonically distinguishable areas were found, showing overlapping projections in at least two of the three experimental animals (Table 1; Figs. 2, 3).

The majority of labeled cells was found ipsilateral to the injection site. Only a few structures showed bilateral labeling. None of the structures showed exclusively contralateral labeling. Those structures containing labeled cells bilaterally always contained more cells ipsilateral than contralateral to the injection site.

Within the telencephalon, medium labeling was found in the rostrodorsal and intermediate parts of the claustrum (Fig. 3, A 19, A 16–14.5). Smaller clusters of labeled cells were found in the basal nucleus of Meynert, substantia innominata, and extended amygdala (Fig. 3, 19–17.5). With the exception of the claustrum, these structures seem to send exclusively ipsilateral projections to the cortical larynx area.

Diencephalic labeling was widespread in the thalamus. Within the ventral and lateral thalamic nuclei group, medium to strong labeling was found in the ventral nucleus ventralis lateralis and the nucleus ventralis posteromedialis, including its parvocellular part (Fig. 3, A 13–11.5, A 8.5–7). Weaker labeling was found in the ventral nucleus ventralis anterior, in both its medial and lateral subnuclei, as well as in the ventral nucleus ventralis posterolateralis (Fig. 3, A 13–11.5, A 8.5–7). Bilateral labeling was only found in the medial subnucleus of the nucleus ventralis lateralis in a single animal.

Within the intralaminar and medial nuclei group, the caudal nucleus medialis dorsalis and the nucleus centrum medianum showed dense labeling (Fig. 3, A 8.5–5.5). Weaker labeling is found in the medial and lateral subnuclei of the nucleus medialis dorsalis, the nucleus intermediodorsalis, nucleus paracentralis, and nucleus parafascicularis (Fig. 3, A 10–5.5). None of these nuclei showed bilateral labeling in more than one animal.

A small to moderate number of labeled cells was also found in the nucleus pulvinaris anterior, field H (Forel) as well as in the posterior and lateral hypothalamic area (Fig. 3, A 11.5, A 5.5). The latter was the only diencephalic region showing bilateral labeling in more than one animal.

In the midbrain and lower brainstem, medium labeling was found in the deep mesencephalic nucleus and median raphe nucleus (Fig. 3, A 8.5, A 2.5). Few labeled cells were found, in addition, in the substantia nigra and medially bordering ventral tegmental area, the dorsal raphe nucleus, oral part of the pontine reticular formation, medial parabrachial nucleus, and locus coeruleus (Fig. 3, A 10–P). In all these structures, labeling was bilateral in at least one animal. No labeled cells were detected below the level of the rostral pons.

DISCUSSION

Subcortical telencephalic input reaches the cortical larynx area from essentially two regions: one is the substantia innominata with its embedded nucleus basalis (Meynert) islands and interdigitating amygdaloid extensions; the other is the claustrum. No input reaches the larynx area from the caudate nucleus, putamen, globus pallidus, and a number of limbic structures, such as the septum, nucleus accumbens, bed nucleus of the stria

Table 1. Subcortical brain structures with output to the cortical larynx area[a]

	Animal I		Animal II		Animal III	
Brain structure	Ipsi	Contra	Ipsi	Contra	Ipsi	Contra
Telencephalon						
Claustrum	++	−	+	−	++	+
Basal nucleus (Meynert)	++	−	+	−	+	−
Substantia innominata	++	−	+	−	+	−
Extended amygdala	+	−	−	−	++	
Diencephalon						
Ventral anterior thalamic nucleus, medial part	++	−	+	−	−	−
Ventral anterior thalamic nucleus, lateral part	+	−	−	−	+	−
Ventral lateral thalamic nucleus, medial part	+	+	+	−	+	−
Ventral lateral thalamic nucleus, lateral part	+++	−	++	−	++	−
Ventral posteromedial thalamic nucleus	+++	−	++	−	++	−
Ventral posteromedial thalamic nucleus, parvocellular part	+++	−	++	−	++	−
Ventral posterolateral thalamic nucleus	+	−	++	−	++	−
Mediodorsal thalamic nucleus, medial part	++	+	++	−	+	−
Mediodorsal thalamic nucleus, caudal part	+++	+	++	−	++	−
Mediodorsal thalamic nucleus, lateral part	+	−	+	−	+	−
Intermediodorsal thalamic nucleus	+	+	−	−	+	−
Centromedian thalamic nucleus	+++	−	++	+	++	−
Paracentral thalamic nucleus	+	−	+	−	−	−
Parafascicular thalamic nucleus	++	+	+	−	+	−
Anterior pulvinar	+	−	+	−	−	−
Field H of Forel	++	−	+	−	−	−
Lateral hypothalamic area	+	+	++	+	−	−
Posterior hypothalamic area	++	−	++	−	−	−
Mesencephalon, pons						
Deep mesencephalic nucleus	+	+	++	+	++	−
Ventral tegmental area	+	−	++	+	−	−
Substantia nigra	−	−	++	+	+	−
Pontine reticular nucleus, oral part	+	−	+	+	+	−
Median raphe nucleus	++	+/−	++	+	−	−
Dorsal raphe nucleus	++	+/−	+	+	−	−
Medial parabrachial nucleus	−	−	++	+	+	−
Locus coeruleus	−	−	+	+/−	+	−

[a] +++ Heavy projection, ++ medium projection, + weak projection, +/− questionable projection, − no projection. Only those structures are listed that contained labeled cells in at least two of the three animals on at least one side. Categorization of labeling is based on the average labeling across all animals.

terminalis, and preoptic region, as well as any olfactory structures. The input from the basal nucleus of Meynert is in agreement with several other studies, which have reported a cholinergic projection from the basal nucleus into large parts of the cortex, an input considered to be important for attention control and memory processes (Mesulam et al., 1983; Richardson and DeLong, 1988). The role of the non-cholinergic projections from the substantia innominata/extended amygdala into the cortex is still unclear.

The claustrum contained two groups of retrogradely labeled cells in the present study: one in its dorsalmost part at the level of the anterior commissure, the other in the intermediate part somewhat more posteriorly. Both regions have been shown in a previous study to also receive input from the cortical larynx area (Simonyan and Jürgens, 2003). The claustrum thus is reciprocally connected with the larynx area. This is in contrast to the caudate nucleus and putamen, which only receive projections from the larynx area, but do not project into it. In fact, the claustrum resembles more the insula in its connections than the striatum: both claustrum and insula are reciprocally connected with the cortical larynx area (Simonyan and Jürgens, 2002, 2004). Consequently, the claustrum rather should be considered as a derivative of the insula than of the striatum.

Thalamic projections into the cortical larynx area arise from motor (VL, VA), sensory (VPM, VPL), associative (MD, APul), intralaminar (CMn, PC, PF) and midline nuclei. All of these nuclei, at the same time, are targets of fibers coming from the cortical larynx area (Simonyan and Jürgens, 2003). While all thalamo-cortical projections of the cortical larynx area are reciprocated, this does not hold for cortico-thalamic projections: the nuclei reticularis, centralis lateralis, centralis medialis, reuniens and pulvinaris medialis and lateralis have been found in our anterograde tracing study to receive fibers from the larynx area, but remained unlabeled in the present study.

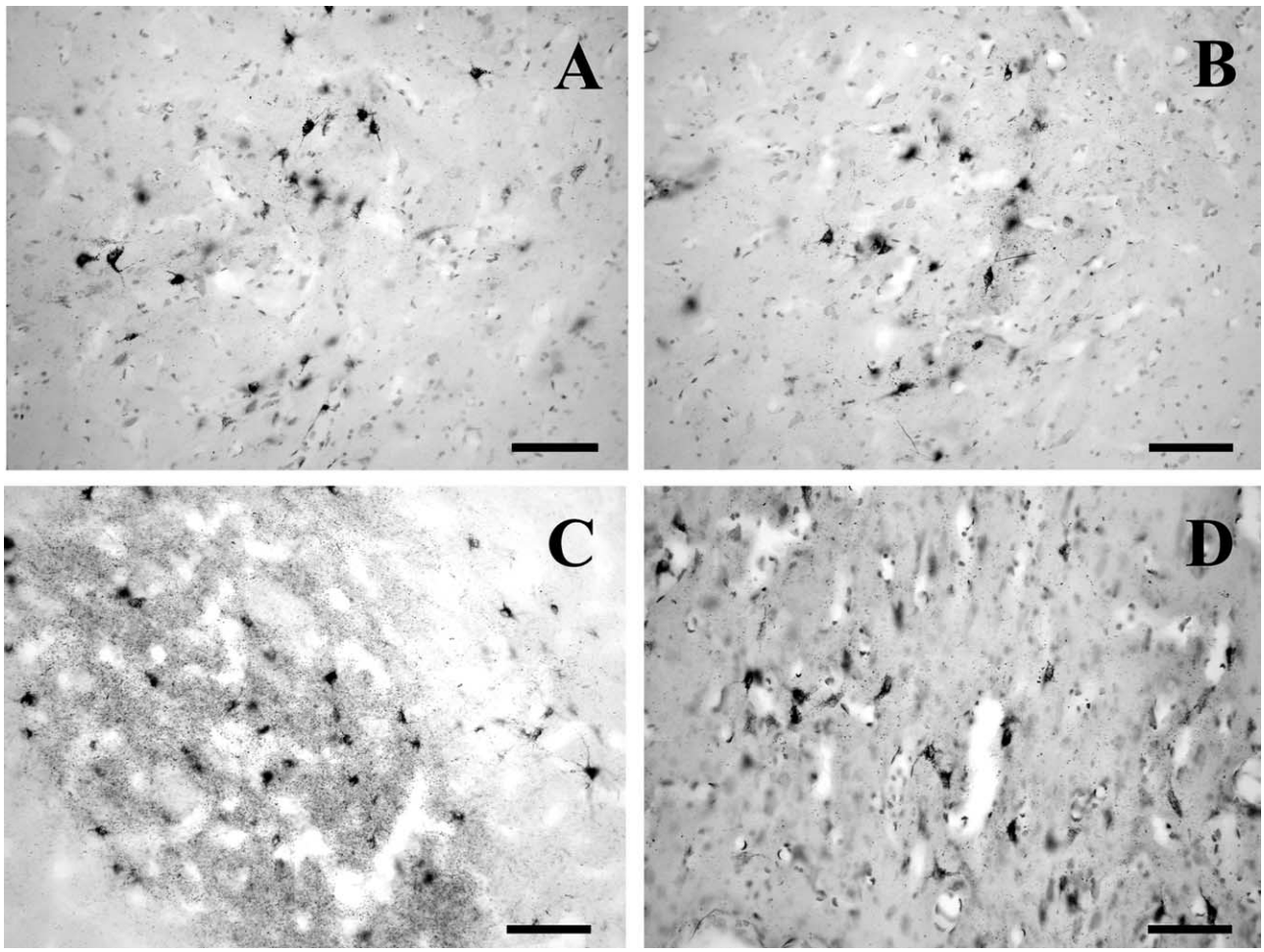

Fig. 2. (A) Microphotographs of retrogradely labeled neurons in the ventral posterolateral thalamic nucleus, (B) ventral posteromedial thalamic nucleus, (C) caudal part of the mediodorsal thalamic nucleus, and (D) intermediodorsal thalamic nucleus. All photographs were taken from animal 1. Scale bars=50 μm.

The nucleus ventralis lateralis usually is considered to be the most important thalamic input nucleus of the motor cortex, providing the latter with information from the cerebellum and basal ganglia (Hoover and Strick, 1999). Single-unit recording studies have revealed a somatotopic organization, with the head represented ventromedially, the legs dorsolaterally and the arms and trunk in between (Vitek et al., 1994). In agreement with the electrophysiological findings, the present study found a massive labeling in the most ventral part of the ventrolateral nucleus. This labeling invaded the medial as well as lateral subnuclei. The strong lateral component in the labeling might be due to the fact that the larynx lies deep in the neck, taking a position between mouth and trunk, and, accordingly, also has its thalamic representation between the more medial mouth representation and the more dorsolateral trunk representation. Recording of the neural activity during vocalization in the cat shows that the ventrolateral thalamus contains numerous neurons with vocalization-correlated activity (Farley, 1997). Brain-imaging studies in humans also have reported phonation-related activity in the thalamus (Bookheimer et al., 1995, 2000; Paus et al., 1996). In these studies, however, the thalamic activity could not be related to the ventrolateral nucleus specifically. Electrical stimulation of the ventrolateral nucleus in humans has been reported to produce vocal utterances (Schaltenbrand, 1975). Lesions invading the ventrolateral thalamus cause dysphonia and dysarthria (Ackermann et al., 1993; Botez and Barbeau, 1971; Schaltenbrand, 1975). These observations suggest that the ventrolateral nucleus of the thalamus plays a crucial role in vocal control by providing the motor cortex with information necessary for the motor coordination of learned vocal utterances.

A massive input reaches the cortical larynx area also from the ventral posteromedial nucleus. This nucleus represents the thalamic relay station for somatosensory information from the face, intraoral cavity, and larynx (Pritchard et al., 1986; Rausell and Jones, 1991). The information reaches the ventral posteromedial nucleus via the spinal and principal trigeminal nuclei as well as the solitary tract nucleus (Beckstead and Norgren, 1979; Beckstead et al., 1980). While the ventral posteromedial nucleus is generally accepted to be the main input structure to the face area of the somatosensory cortex, the present study makes clear that also parts of the motor

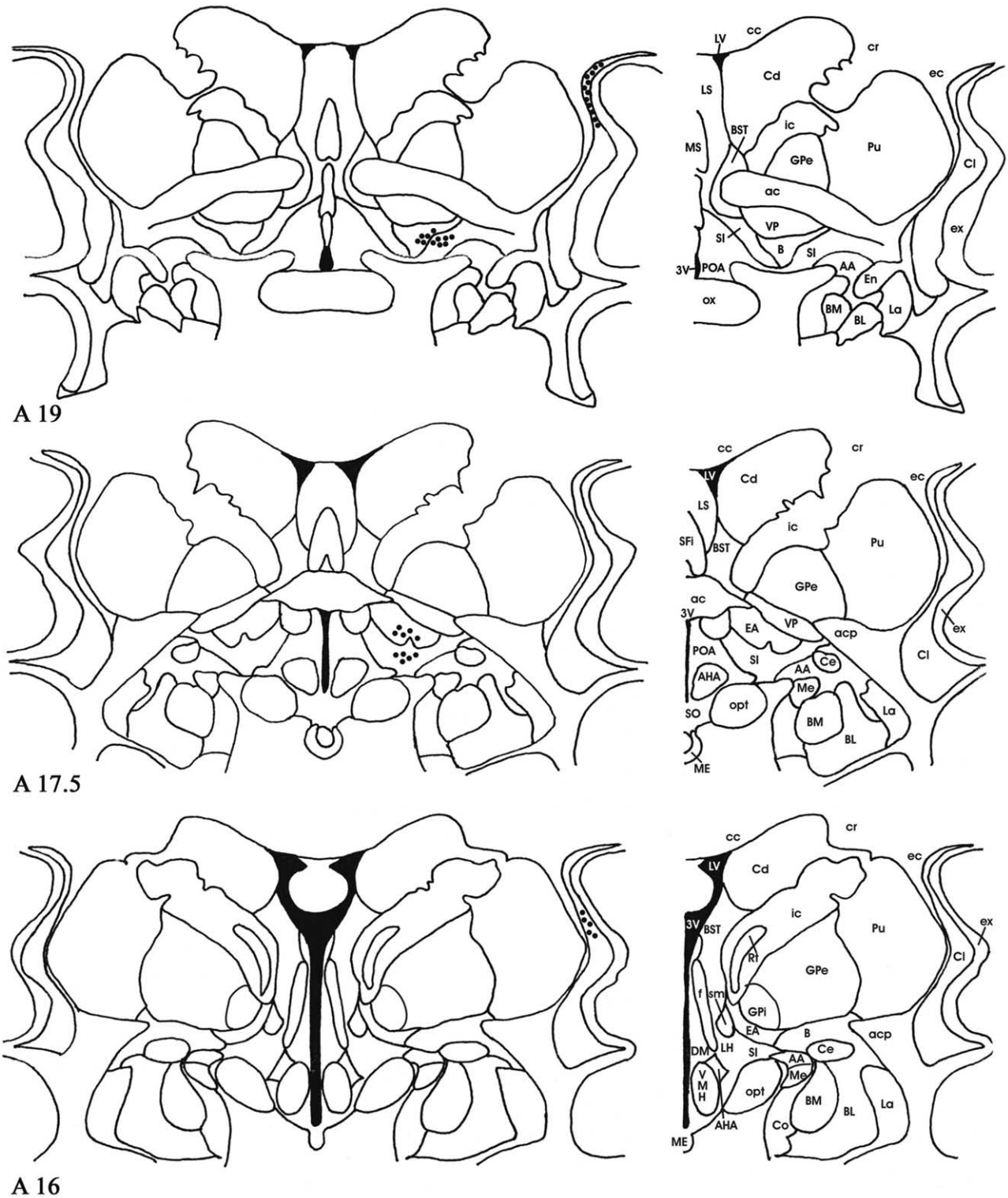

Fig. 3. Brain diagrams of the rhesus monkey in the frontal stereotaxic plane, showing retrograde projection areas overlapping in at least two of the three experimental subjects. Dots indicate regions of retrogradely labeled neurons.

cortex are directly connected with this nucleus. This means that the motor cortical larynx area is able to gain information about the somatosensory status of the phonatory apparatus—at least to some extent—without somatosensory cortex. Somatosensory (including proprioceptive) information from the phonatory apparatus

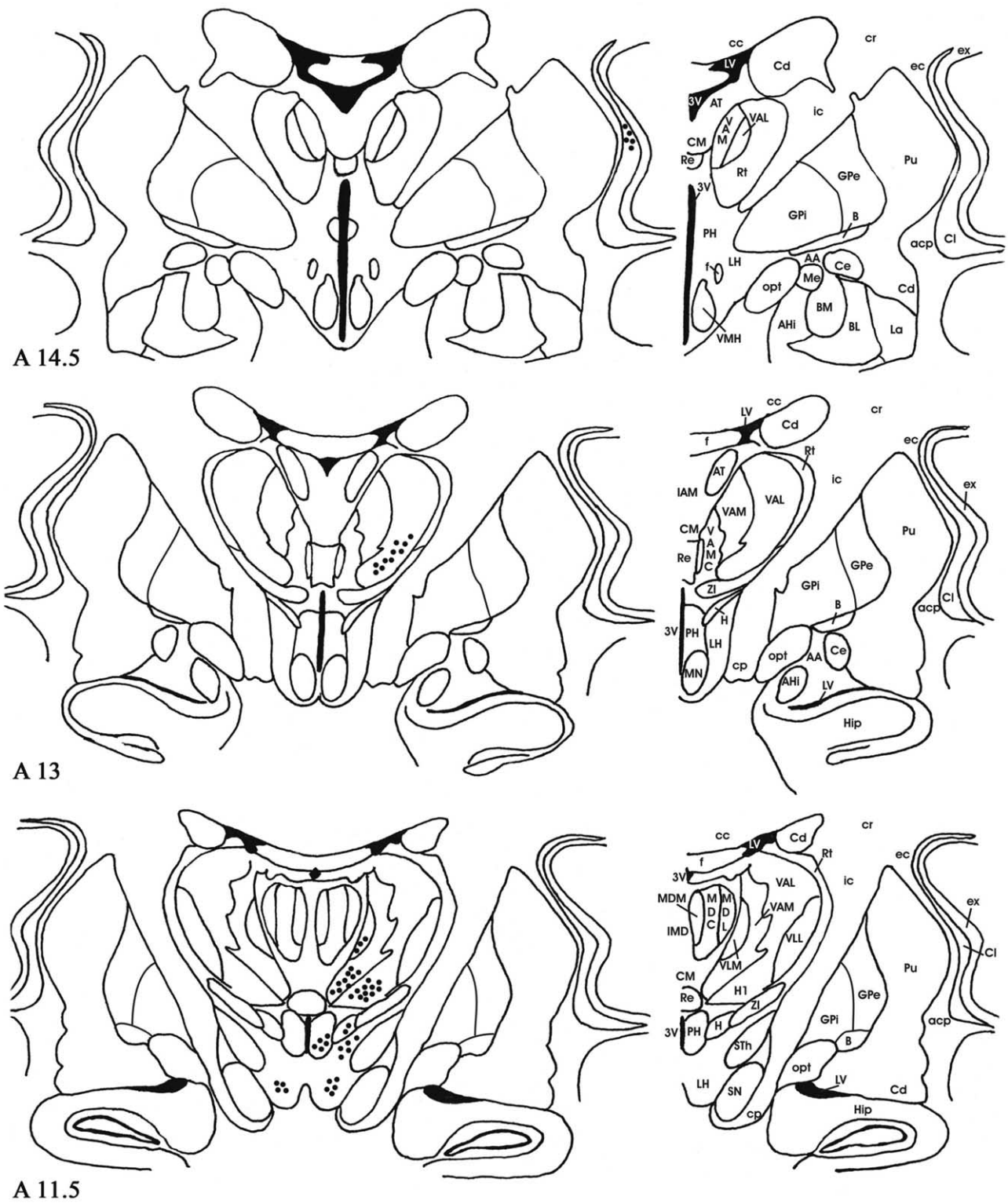

Fig. 3. (*Continued*).

seems to be essential in phonation control. Interruption of somatosensory feedback from the larynx by cutting the internal laryngeal nerve or lesioning the solitary tract nucleus causes dysphonia in monkeys (Jürgens and Kirzinger, 1985; Thoms and Jürgens, 1981). Electrical stimulation of the ventral posteromedial nucleus during vocalization affects the acoustic structure of monkey calls (Dressnandt and Jürgens, 1992). Single-unit re-

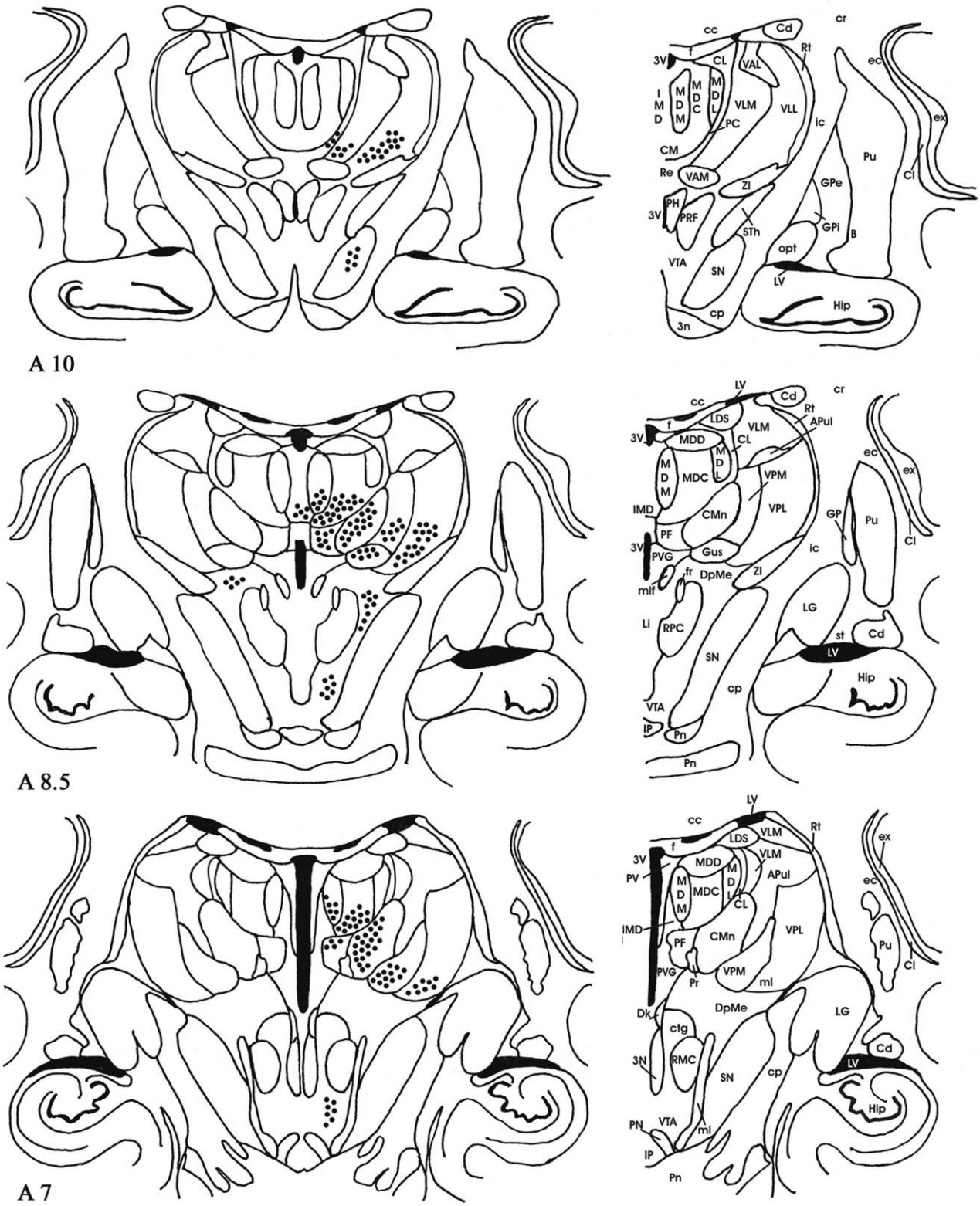

Fig. 3. (*Continued*).

cording in the human ventral posteromedial nucleus has demonstrated speech-related neuronal activity (McClean et al., 1990). The ventral posteromedial nucleus thus seems to provide the motor cortical larynx area with somatosensory information from the mouth and larynx region, necessary for vocal motor coordination.

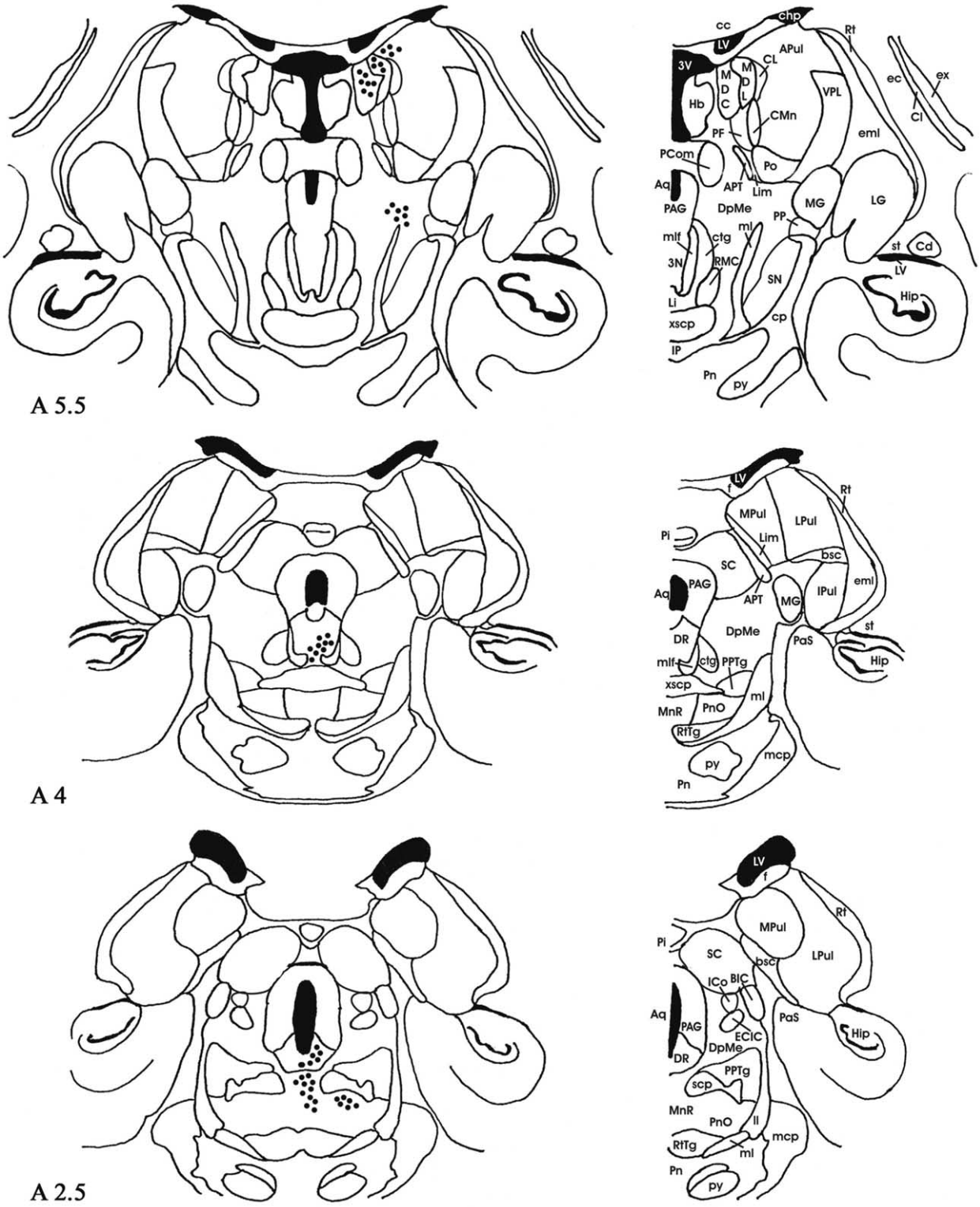

Fig. 3. (*Continued*).

Thalamic input reaches the cortical larynx area also from the nuclei ventralis anterior, medialis dorsalis and pulvinaris anterior. All three inputs are atypical for a motor cortical area, but typical for the premotor cortex (Matelli et al., 1989; Rouiller et al., 1999). These inputs thus reflect the fact that the larynx area occupies an extremely rostral position within the motor cortex, lying cytoarchitectonically in area 6, instead of area 4.

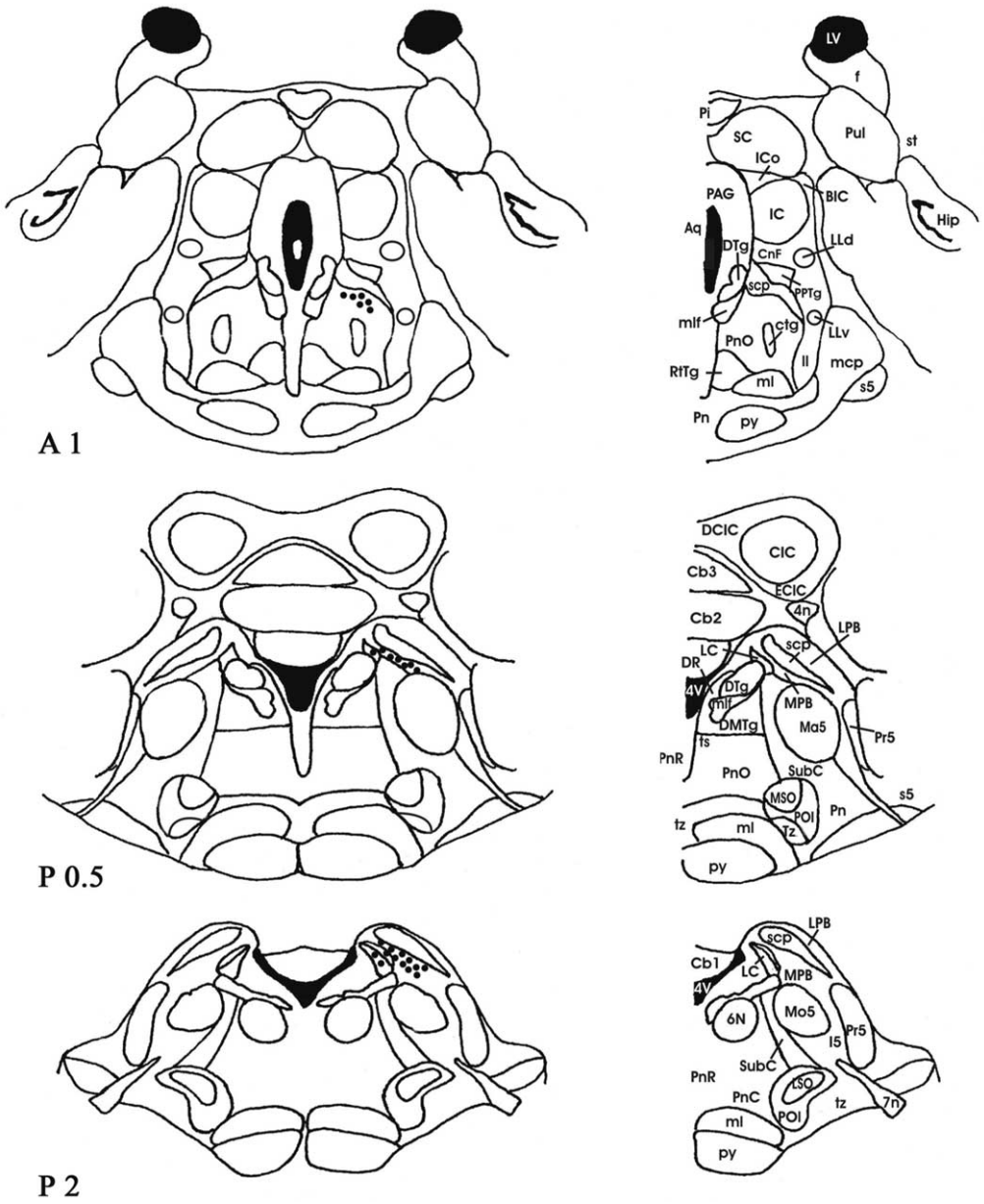

Fig. 3. (*Continued*).

A diencephalic input contributing only in a very indirect way to vocal behavior comes from the lateral and posterior hypothalamus. Electrical stimulation of these regions in the squirrel monkey has been reported to produce vocalization (Jürgens and Ploog, 1970). But these vocalizations could be shown to represent secondary reactions to stimulation-induced emotional effects (Jürgens, 1976). Accordingly, the hypothalamic projection may be interpreted as a pathway by which emotional influences gain access to the cortical larynx area.

In the mesencephalon, essentially two groups of retrogradely labeled neurons were found. One was located in the deep mesencephalic nucleus, extending into the oral pontine reticular formation. This region has been found in our previous study also to receive projections from the cortical larynx area (Simonyan and Jürgens, 2003). It thus represents part of reticular formation reciprocally connected with the larynx area. In contrast to this bi-directional, weak connection, there exists also a very massive connection with the reticular formation. This massive projection connects the cortical larynx area with the dorsal and lateral medullary reticular formation, and is exclusively corticofugal (Simonyan and Jürgens, 2003).

The second mesencephalic group of retrogradely labeled neurons was found in the ventral tegmental area and substantia nigra. The group consisted of a small number of scattered cells. These cells probably represent predominantly dopaminergic neurons of the A8–A10 complex, which have been shown to project directly into the motor

cortex (Gaspar et al., 1992; Williams and Goldman-Rakic, 1998).

In the pons, apart from the reticular formation, three areas maintain direct connections with the cortical larynx area. One is the locus coeruleus. Similar to the dopaminergic ventral tegmental/substantia nigra projection, the locus coeruleus provides the cortex with a diffuse noradrenergic input (Foote, 1997; Moore and Bloom, 1979). This input seems to be important for selective attention processes (Grant et al., 1988; Berridge and Waterhouse, 2003; Segal, 1985). The retrogradely labeled cells found in the dorsal and median raphe nuclei represent a third monoaminergic diffuse input into the cortex. In this case, serotonin is the acting transmitter. Its specific role in motor cortical functions is still unclear (Azmitia and Gannon, 1986; Berger et al., 1988; Consolazione and Cuello, 1982; Wilson and Molliver, 1991).

A third pontine structure projecting to the cortical larynx area is the medial parabrachial nucleus. In contrast to the locus coeruleus and raphe nuclei, it is connected with the larynx area reciprocally (Simonyan and Jürgens, 2003). The medial parabrachial nucleus receives its input mainly from the rostral and medial solitary tract nucleus (Herbert et al., 1990), which itself represents the main relay station for intraoral and laryngeal somatosensory information (Hayakawa et al., 2001; Patrickson et al., 1991; Travers and Norgren, 1995). The medial parabrachial nucleus, apart from its direct connection to the cortical larynx area, also has an indirect connection with it via the ventral posteromedial thalamic nucleus, which, in the present study, has been shown to be one of the main input structures of the cortical larynx area (Pritchard et al., 2000; Saper and Loewy, 1980). Single-unit recordings in the cat have revealed vocalization-correlated neuronal activity in the parabrachial area, including the medial parabrachial nucleus (Farley et al., 1992). These findings suggest that the medial parabrachial nucleus, similar to the ventral posteromedial nucleus, is involved in vocal motor coordination by providing the cortical larynx area with proprioceptive and tactile information from the larynx and oral cavity.

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