Vocalization-correlated single-unit activity in the brain stem of the squirrel monkey*

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Summary. The brain stems of 17 squirrel monkeys (Saimiri sciureus) were systematically explored for vocalization-related single-unit activity during calls electrically elicited from the periaqueductal grey. Of 12,280 cells tested, 1151 fired in relation to vocalization. Of these, 587 reacted to external acoustic stimuli and started firing after vocalization onset. As most of these cells were located in classical auditory relay structures, they probably represent auditory neurones reacting indirectly to self-produced vocalization due to auditory feedback. Seven cells reacted to acoustic stimuli but fired in advance of self-produced vocalization. These cells were located in the pericentral inferior colliculus, dorsal nucleus of the lateral lemniscus, dorsomedial to the ventral nucleus of the lateral lemniscus and immediately lateral to the central grey. They are probably engaged in tuning the auditory system to process self-generated sounds differently from external sounds. 261 neurones reacted to nonphonatory oral movements (chewing, swallowing) and started firing after vocalization onset. These neurones were widely distributed within the brain stem, with the highest density in the spinal trigeminal nucleus and medially adjacent reticular formation. The majority of these cells seem to react to proprioceptive and tactile stimuli generated by phonatory and nonphonatory oral activities. Some of them may exert motor control on muscles that come into play at later stages of phonation. 57 neurones reacted to nonphonatory oral movements but fired in advance of vocalization onset. These neurones were located mainly in the trigeminal motor nucleus, nucleus ambiguus, reticular formation around these nuclei, parabrachial region and lateral vestibular nucleus. Their role in motor control seems to be related to specific muscles rather than specific functions. 100 of the vocalization-related cells showed a correlation with respiration. Expiration-related cells were found in and around the rostral nucleus ambiguus and in the reticular formation dorsal to the facial nucleus. Inspiration-related cells were located in the rostral and caudal nucleus ambiguus regions, ventrolateral solitary tract nucleus and the lateral reticular formation below the trigeminal motor nucleus. Most of these cells probably represent premotor neurones of respiratory muscles and laryngeal motoneurones of the cricothyroid and posterior cricoarytenoid muscles. Finally, a last group of cells was found that was unresponsive to chewing and swallowing movements, quiet breathing and acoustic stimuli, but changed activity during vocalization. 38 of them became active before vocalization and cricothyroid activity, and 101 afterward. Both types were completely intermingled and scattered widely in the brain stem, including the nucleus ambiguus region, solitary tract nucleus, nucleus reticularis parvocellularis and gigantocellularis, parabrachial region, pericentral colliculus inferior, vestibular complex, periventricular grey and laterally adjacent tegmentum. Some of these cells may be related to vocalization in a more specific way.

Abbreviations: A nucl. annularis; Ab nucl. ambiguus; Apt area praetectalis; BC brachium conjunctivum; BP brachium pontis; Cb cerebellum; CC corpus callosum; Cd nucl. caudatus; Col colliculus inferior; CoS colliculus superior; CRF corpus restiforme; DBC decussatio brachii conjunctivi; DG nucl. dorsalis tegmenti (Gudden); DR nucl. dorsalis raphae; DV nucl. ventralis n. vagi; FRM formatio reticularis myelencephali; FRP formatio reticularis pontis; FRTM formatio reticularis mesencephali; GC substantia grisea centralis; GPM griseum periventriculare mesencephali; GPo griseum pontis; H habenula; Hip hippocampus; IP nucl. interpeduncularis; LC locus coeruleus; LL lemniscus lateralis; Lld nucl. dorsalis lemnisci lateralis; LLv nucl. ventralis lemnisci lateralis; LM lemniscus medialis; LP nucl. lateralis posterior thalami; MD nucl. medialis dorsalis thalami; MV nucl. motorius n. trigemini; NC nucl. cochlearis; NBC nucl. cerebelli; NCS nucl. centralis superior; NCT nucl. trapezoidalis; NR nucl. raber; NST nucl. supratrochlearis; NSV nucl. spinalis n. trigemini; NTS nucl. tractus solitarii; NIH nucl. oculomotorius; NIV nucl. trochlearis; nV nervus trigeminus; NVI nucl. abducentes; NVII nucl. facialis; NXII nucl. hypoglossus; OL oliva inferior; Pbl nucl. parabrachialis lateralis; PbM nucl. parabrachialis medialis; Pnu nucl. praepositus; Pu nucl. pulvinaris oralis; PuL nucl. pulvinaris lateralis; PuM nucl. pulvinaris medialis; Py tractus pyramidalis; PV nucl. principalis n. trigemini; RL nucl. reticularis lateralis; RTP nucl. reticularis tegmenti pontis; SN substantia nigra; ST stria terminalis; Ves nucl. vestibulares; VR nucl. ventralis raphae; IV decussatio n. trochlearis

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The aim of the present study was to determine which brain areas are involved in the production of vocalization. For this purpose, a systematic exploration of the squirrel monkey's brain was undertaken in an attempt to locate vocalization-related single-unit activity. Up to now only very few single-unit recording studies have dealt with vocalization-related activity. The most thorough studies stem from Larson and Kistler (1984, 1986) in macaques. In these studies, however, the recording sites were limited to the periaqueductal grey of the midbrain. This region was also the focus of a study on echolocation sound-related neuronal activity in bats (Suga and Yajima 1988). In bats, in addition, a detailed analysis was made in the nucleus ambiguus by Rübsamen and Betz (1986). This region was also investigated by Yajima et al. (1982) in the rat. Furthermore, there are two reports on vocalization-related activity in the anterior cingulate cortex by Sutton et al. (1978) in macaques and by Müller-Preuss (1988) in the squirrel monkey. The present study thus is the first in which an extensive unbiased mapping of large brain areas for vocalization-related activity has been undertaken.

Other techniques that have been used to identify vocalization-controlling brain structures are electrical and chemical brain stimulation, lesioning and neuroanatomical tracing (for a review see Sutton and Jürgens 1988). Each of these techniques has its drawbacks as well as its advantages. Brain stimulation enables artificial activation of localized neuronal circuits. As it cannot reproduce natural activity patterns (at least not at the site of stimulation), one cannot be sure that an area normally engaged in vocal control does yield vocalization under brain stimulation. On the other hand, some brain structures yield vocalizations when stimulated but obviously do not represent vocalization-controlling structures in a more strict sense. In other words, there is the problem of distinguishing between directly elicited vocalizations and those representing secondary responses due to stimulation-induced sensory or emotional effects (in the sense that a shriek results from a painful stimulus). Lesioning studies have probably been the most informative approaches in the past - starting with the discovery of the motor speech cortex by Paul Broca in 1861. Lesioning experiments, however, are very animal-consuming, thus precluding a comprehensive mapping study in higher mammals. Furthermore, if there is a lesion-induced change in vocal behaviour, we still usually do not know whether this change is due to interruption of fibres of passage or destruction of vocal relay stations. Finally, neuroanatomical studies have been fruitful in delimiting the motoneurone pools that innervate the phonatory muscles, in tracing their afferent input, and in determining the efferent connections of the primary sensory relay nuclei involved in phonation. The interpretation of higher order projections, however, requires functional methods and cannot be made on a neuroanatomical basis alone. The present study is thus meant as a complementary approach, closing some of the gaps left by the other techniques.

Methods

In 17 squirrel monkeys (Saimiri sciureus), midbrain, pons and medulla were systematically explored with roving microelectrodes for neurones showing vocalization-related activity. For this purpose, a recording chamber made of macrolon (outer diameter 20 mm, inner diameter 16 mm) was implanted stereotactically onto the skull under general anaesthesia (40 mg/kg pentobarbital sodium). The chamber was fixed with the aid of five stainless steel screws anchored in the skull with nuts and dental cement. The bone underneath the chamber lumen was removed with a dental drill. The chamber contained a small platform with electrode guides (0.5 mm i.d.) through which, in a later step, stimulation electrodes could be introduced into the brain. These stimulation electrodes were used to elicit vocalization electrically. They were implanted into the periaqueductal grey of the midbrain, an area in the squirrel monkey known to produce vocalization readily when electrically stimulated (Jürgens and Ploog 1970). The stimulation electrodes consisted of a stainless steel tube (0.47 mm o.d.) containing a teflon-covered stainless steel wire (ø0.12 mm), uninsulated at the tip for 1 mm and protruding from the tube for 2 mm. The recording electrodes were purchased from Frederick Haer and AM Systems Inc. and consisted of lacquer-insulated stainless steel and tungsten microelectrodes with an impedance of 8–12 MΩ (at 1,000 Hz) and a shaft diameter of 0.6 and 0.35 mm, respectively. The EMG electrodes were implanted into the temporalis and cricothyroid muscles of one side. The temporalis electrode consisted of a teflon-insulated stainless steel wire (ø0.12 mm) with an uninsulated tip of 0.5–1 mm. The cricothyroid electrode was made of a multi-strand teflon-coated wire (0.15 mm. AM Systems Inc.) with an uninsulated tip of 0.1–0.2 mm. Both EMG electrodes were led subcutaneously to a rectangular macrolon platform attached to the outer surface of the recording chamber. There they ended in a Winchester connector together with a wire coming from the five fixation screws and serving as the indifferent electrode.

One week after implantation of the recording chamber and EMG electrodes, the animal was placed in a monkey chair and implantation of stimulation electrodes took place. The electrodes were lowered through the guiding tubes into the periaqueductal grey in 1 mm steps. After each step, the elicitability of vocalization was tested with biphasic rectangular pulses of 1 msec duration, 30 pulse pairs per second, 40–320 μA peak current and train durations of 5–15 s. After a position was found where the animal emitted repetitive calls in a relaxed way, the electrode was fixed, and one day later probing with microelectrodes began. Microelectrodes were carried by a David Kopf microdrive (607-C) attached to an x-y-micropositioner and mounted on the recording chamber. Microelectrodes were lowered through the whole depth of the brain stem in steps of 50–100 μm. X-ray photographs were taken from the initial recording position, the lowest position and positions of special interest. For this purpose, the animal's head was fixed by the platform attached to the recording chamber in a standardized way so that X-ray photographs in the stereotactic frontal and sagittal planes could be taken. In this way, the relative position of the microelectrode to the chronically implanted stimulation electrodes could be determined. In some cases electrolytic lesions were made at the end of a session by passing ca. 30 μA of direct current through the electrode to mark recording sites of interest.

The recorded signals were led through a preamplifier (BAK A-1B) and filters (Kemo VBF/4 and Neurolog NL 115) to an artefact suppressor (custom-made). The artefact suppressor short-circuited the recording line for the duration of the stimulation artefact, which lasted 5 to 8 ms, depending upon the stimulation intensity and the...