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SHORT COMMUNICATION
Localization of a vocal pattern generator in the pontine brainstem of the squirrel monkey

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Abstract

Very little is known about the coordination of muscles involved in mammalian vocalization at the level of single neurons. In the present study, a telemetric single-unit recording technique was used to explore the ventrolateral pontine brainstem for vocalization-correlated activity in the squirrel monkey during vocal communication. We found a discrete area in the reticular formation just above the superior olivary complex showing vocalization-correlated activity. These neurons showed an increase in neuronal activity exclusively just before and during vocalization; none of them was active during mastication, swallowing or quiet respiration. Furthermore, the neuronal activity of these neurons reflected acoustic features, such as call duration or syllable structure of frequency-modulated vocalization, directly. Based on these findings and previously reported anatomical data, we propose that this area serves as a vocal pattern generator for frequency-modulated call types.

Introduction

Monkey calls can be considered as homologous to non-verbal emotional vocal utterances of humans, like laughing, crying or moaning. Accordingly, they have been used as models to study the neurobiological processes underlying emotional vocal communication (Jürgens, 1986). While the muscles involved in phonation and their motoneurons have been characterized in detail, as well as the centres and pathways of the forebrain and midbrain controlling the elicitation of vocalizations, very little is known about how the phonatory motoneuron pools are coordinated to accomplish a specific vocal pattern (Jürgens, 2002). The ventrolateral pontine brainstem (VLPB) is a candidate region for the vocal-motor pattern generator: the VLPB has direct connections to all cranial motoneuron pools involved in phonation (Hannig & Jürgens, 2005); stimulation of the VLPB yields vocalization (Jürgens & Ploog, 1970; de Lanerolle, 1990; Behrend & Schuller, 2000), and blocking of its excitatory neurotransmission eliminates specific call types electrically elicitable from the periaqueductal grey (PAG) of the midbrain (Jürgens, 2000).

In the present study, we explored the VLPB with a previously developed and recently modified telemetric recording technique, which allows simultaneous recording of extracellular single-unit activity and spontaneously uttered vocalizations in freely moving squirrel monkeys (*Saimiri sciureus*) within their social group (Grohrock *et al*., 1997; Jürgens & Hage, 2006).

Materials and methods

Animals and surgery

This study was carried out in three male squirrel monkeys (*Saimiri sciureus*), aged 3 years at the beginning of the experiments. Surgical anaesthesia was induced with ketamine (30 mg/kg body weight) and xylazine (6 mg/kg) intramuscularly; a prolonged anaesthetic state was maintained by repeating doses of ketamine and xylazine (injections with half and quarter initial dosage).

The animals were placed in a stereotaxic apparatus and a platform with numerous steel guiding tubes was implanted by the aid of four stainless steel screws, anchored in the skull with nuts and embedded in dental acrylic (Paladur, Kulzer, Wehrheim, Germany). After 4 weeks of recovery, custom-made microdrives were mounted on the platform under general anaesthesia (see above), allowing the dorsoventral exploration of two microelectrodes at the same time over a distance of 8–10 mm in 50–100-μm steps, in the freely moving animal. Electrodes were positioned stereotaxically and recording sites were verified immunohistologically at the end of the experiments according to Benevento & McCleary (1992). Histological evaluation was made with the aid of the stereotaxic atlas of Emmers & Akert (1963).

Telemetric recording procedure

The neuronal activity picked up by the two electrodes was amplified by a MOSFET operational amplifier and fed into a custom-made transmitter circuit each (for details of the electronic circuitry and telemetry setup, see Grohrock *et al*., 1997). An additional transmitter was used to send the signal of a piezo-ceramic skull vibration sensor mounted on the platform. This sensor served to distinguish the vocalizations of the experimental animal from those of the other animals of the group by comparing the signals coming from the room microphone and the skull vibration sensor. The whole headstage, including platform, microdrive, amplifiers, transmitters and protection cap, had a weight of 32 g (Jürgens & Hage, 2006). Transmitters were powered with two rechargeable lithium batteries. The recording session took place in a cage with dimensions 2.4 × 0.8 × 0.8 m, in which the experimental animal was housed with one or two other animals. Two additional groups of three squirrel monkeys each were held in the same room with visual, acoustic

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and restricted tactile contact with the experimental animal. The room was lined with foam rubber mats to reduce acoustic reflections.

The transmitter signals were picked up by two interconnected, orthogonally arranged antennae (length: 2.4 m and 1.2 m) within the animals' cage. The antenna signal was preamplified (Conrad, PA-21, Hirschau, Germany) and sent via a coax-cable to three receivers (Yaesu, VR-5000, Cypress, CA, USA). After demodulation, the telemetric signals were sent to a four-channel video recorder (JVC, BR-S611E, Friedberg, Germany) for long-term storage and to a personal computer (Pentium IV, 2 GHz) via an analogue/digital interface (CED, Micro 1401 mkII, Cambridge, UK) for data analysis. Additional signals reached the video recorder from a video camera, installed in the animal room. The signal was displayed on a monitor (Panasonic, TC-1470Y, Hamburg, Germany) and served for continuous observation of the experimental animals from the central lab. Furthermore, the signal of a microphone (Sennheiser, ME 64 + K6, Wedemark, Germany) placed in the animal room was sent via a microphone preamplifier (M-Audio, Audio Buddy, Oehringen, Germany), an audio amplifier (Digitimer, NL 120, Hertfordshire, UK) and a high pass filter (Digitimer, NL 125, Hertfordshire, UK; cut-off frequency 300 Hz) to the video recorder and the PC via the above-mentioned analogue/digital interface.

Recording sessions lasted 10–15 min and were made twice per day during feeding time, because the frequency of vocalization was the highest during that time. Neuronal activity was tested during all call types uttered. Quantitative data analysis was performed for two highly frequency-modulated call types with a specific repetitive character (trill, cackle) and a low-pitched non-rhythmic call (caw). Examples of these call types are depicted in Fig. 2A. To differentiate between vocalization-correlated neurons and auditory neurons, which are also active during the animal's own vocalization, each recorded position was tested, in addition, with vocalizations uttered by the group mates and auditory stimuli presented from a speaker (white noise, 0 Hz–200 kHz, 80 dB SPL 300 ms duration including 10 ms rise and fall times). White noise bursts were used, as almost all auditory neurons in the pontine brainstem show responses to this kind of stimulus (i.e. Caird & Klinke, 1983; Irvine & Jackson, 1983).

Data analysis was carried out using the software Spike 2, Version 5 (CED). For the identification of vocalization-correlated and auditory units, conventional peri-event time histograms and peri-stimulus time histograms, respectively, were constructed after having submitted the original recording to a spike-sorting procedure (template-based spike-clustering). To avoid measuring the same unit at two consecutive positions, only the largest spike form recorded at a specific position was used for data analysis.

Statistical analyses were performed by using the chi-square test when comparing distributions of neuronal properties in different brain areas. The Pearson's correlation was used to examine the correlation between call duration and the duration of neuronal activity. All tests were made with SPSS 12.0.1 (SPSS, Chicago, USA). Correlations and differences in distributions were considered significant if the probability of error was less than 5%.

The experiments were approved by the Animals' Ethics Committee of the district government Braunschweig, Lower Saxony, Germany. The experiments conformed to the NIH guidelines on the ethical use of animals, and care was taken to minimize the number of animals used.

Results and discussion

In three squirrel monkeys, we recorded from 325 positions along 15 electrode tracks in the VLPB during self-produced vocalization, feeding behaviour and auditory stimulus presentation. Our main focus was the reticular formation outside the phonatory motoneuron pools. At 203 positions, single units could be isolated. Of these, 19 neurons showed vocalization-correlated activity (Fig. 1A–C), 19 were active during mastication (Fig. 1A–C) and 24 responded to external auditory

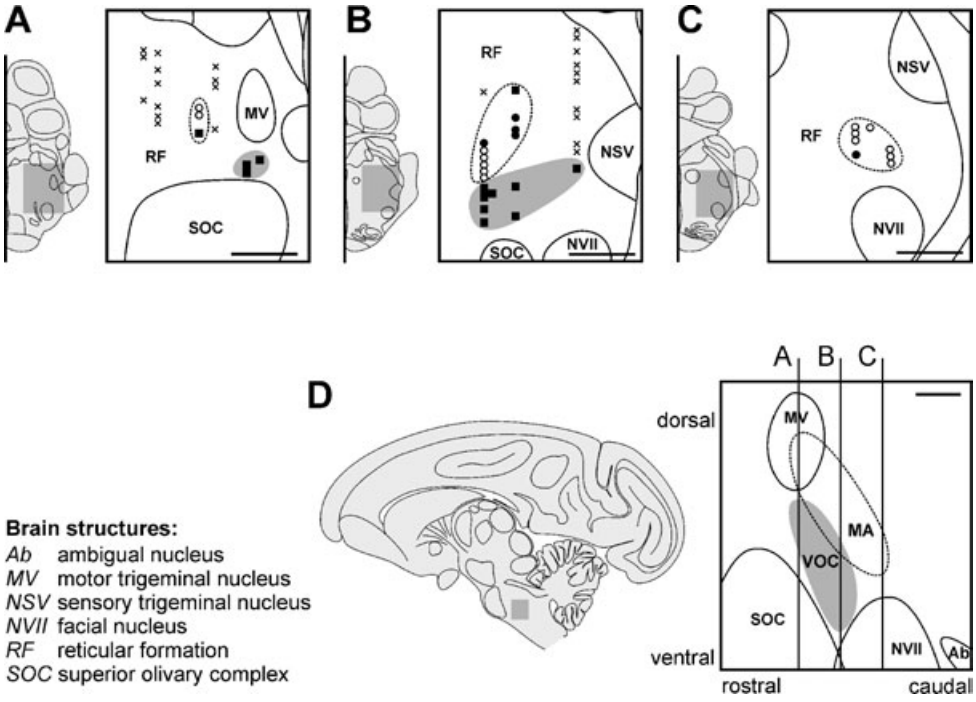

FIG. 1. Frontal and sagittal views of the squirrel monkey VLPB. (A–C) Locations of purely vocalization-correlated neurons (filled squares), vocalization- and mastication-correlated neurons (filled circles), purely mastication-correlated neurons (open circles) and auditory neurons (crosses). (D) Sagittal section (lateral 1.8–3.8 mm) indicating the position of the frontal sections (A–C) and the position of MA and VOC, according to frontal sections. Scale bar, 500 μm.

TABLE 1. Distribution of vocalization- and/or mastication-correlated neurons in the VOC and MA groups

	VOC	MA
Neurons only active during vocalization	12 (10)	2 (0)
Neurons active during vocalization and mastication	0	5 (2)
Neurons only active during mastication	0	14

MA, group of neurons showing neuronal activity to vocalization and to mastication; VOC, group of neurons, showing neuronal activity exclusively to vocalization. The numbers in brackets indicate neurons showing a significant correlation to call duration and/or a clear relationship to syllable structure.

stimuli (Fig. 1A and B). The anatomical position and physiological response of the auditory neurons is in agreement with earlier studies and will not be discussed here (Irvine & Jackson, 1983). Most of the vocalization-correlated neurons were only active during vocalization, without additional activity during mastication, swallowing or quiet respiration (14/19). Five neurons showed additional activity during mastication. None of the vocalization-correlated neurons reacted to auditory stimuli.

Using anatomical position, physiological data and statistical criteria, we divided vocalization-correlated neurons into two groups. For a better overview, Table 1 summarizes the distribution of neurons with vocalization- and/or mastication-correlated activity within the two groups that are defined below.

The first group (12 neurons), directly dorsal to the superior olivary complex, showed an increase in activity exclusively just before and during vocalization; none of them changed activity during mastication or quiet respiration (VOC, Fig. 1A, B and D). In 10 of them, firing was related either to call duration (4/12), syllable structure (1/12) or both (5/12, Fig. 2D–E). All nine neurons showing call duration-correlated neuronal activity had *P*-values of less than 0.05 (8 out of 9 had *P*-values less than 0.01) and *r*-values between 0.84 and 0.98. An

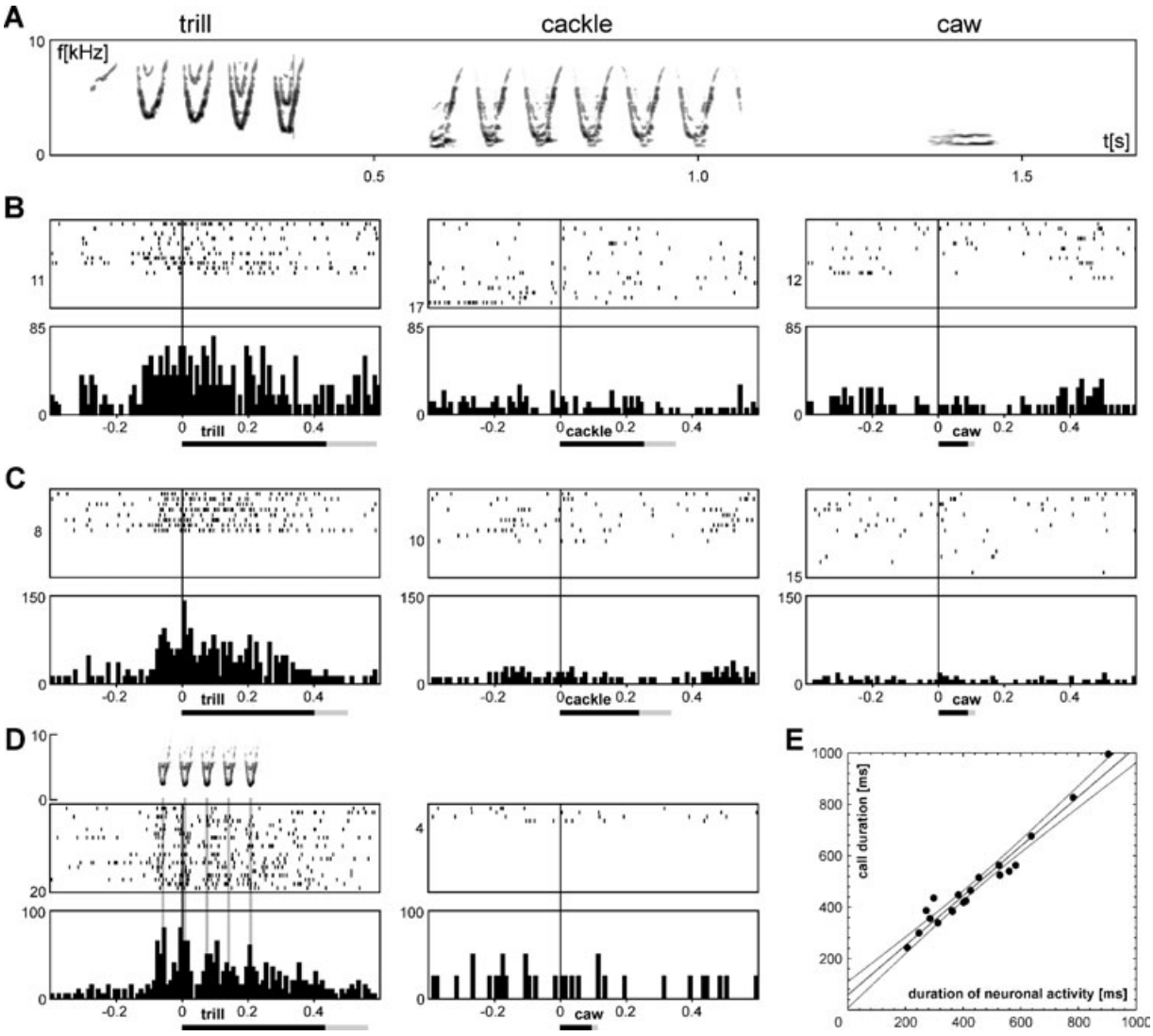

FIG. 2. Neuronal activity of three VOC neurons (B–D) during self-produced trill, cackle and caw calls. (A) The sonograms of the three call types, as recorded with the bone vibration sensor. (B–D) The neuronal activity as raster (top panels) and peri-event time histograms (bottom panels). The black bars below the histograms indicate the onset and mean duration of vocalizations; the grey bars indicate standard deviation. (B and C) Neurons showing vocalization-correlated activity during trill, but not during cackle or caw calls. (D) Neuron showing trill syllable-correlated activity, but no activity during caw. Grey vertical lines relate the maxima of neuronal activity to the corresponding syllables of a representative trill call. (E) Same neuron as in (D), showing statistically significant correlation between call duration and duration of neuronal activity (Pearson's correlation, $P < 0.001$, $r = 0.97$; each dot stands for one trill call). Different call durations are mainly due to different numbers of syllables in trill/cackle calls.

example for a significant correlation between call duration and the duration of neuronal activity is shown as a scatterplot in Fig. 2E. Neurons showing a syllable-correlated activity could be easily distinguished from neurons having no correlated activity by their clear correlation between syllable rate and the distance between the maxima in neuronal activity, as shown in Fig. 2D. The median time between two consecutive syllables was 70.3 ± 1.0 ms and the median time between consecutive maxima in neuronal activity was 70.5 ± 0.8 ms, indicating a close correlation between syllable structure and neuronal activity.

The measured latencies of VOC neurons were in a range, which encompassed nearly the complete range of the latencies of the phonatory motoneuron pools tested (18–49 ms). All neurons showed vocalization-correlated activity only to frequency-modulated call types, such as trill and cackle, some even exclusively to trill calls (Fig. 2B and C); none was active during caw.

The activity of the second group was vocalization- and mastication-correlated. This group was located dorsal to VOC and formed part of a larger area (MA, Fig. 1A–D) containing, in addition, purely mastication-correlated neurons (14/21). Vocalization-correlated MA neurons showed significant differences to VOC neurons in firing properties and call pattern correlation (chi-square test, $P < 0.05$, $n = 19$). While all VOC neurons showed activity before and during vocalization, less than half of the neurons in MA did so (3/7); some were active only during (2/7), after (1/7) or before, during and after vocalization (1/7). Only two MA neurons showed a significant correlation between call duration and neuronal activity (Pearson's correlation, $P < 0.05$, $r = 0.43$ and 0.89); none fired in correlation to syllable structure.

Because almost all MA neurons show mastication-correlated activity (19/21), we suggest that these neurons are part of the masticatory pattern generator. Such a generator has been described already for non-primates; its position is identical with MA of the present study (Lund *et al*., 1998; Nakamura *et al*., 2004). We suggest that the vocalization-correlated activity of MA neurons serves as feedforward- or feedback-information from motor or sensory structures, respectively, to avoid interference between masticatory and vocal motor commands. This interpretation is supported by the finding that only MA neurons (2/7) and no VOC neurons showed vocalization-correlated inhibition.

Our findings point to a vocal pattern generator for frequency-modulated vocalization in the VLPB dorsal to the superior olivary complex. Neurons in VOC were only active during frequency-modulated vocalizations. This fits with a previous study, in which only frequency-modulated calls could be blocked after a glutamate-antagonist injection into the VLPB (Jürgens, 2000).

In earlier studies (Holstege, 1989; Larson, 1991), the PAG has been proposed to contain the pattern generator for non-verbal emotional vocalization in general. However, in the light of more recent studies this seems unlikely because, in contrast to VOC (Mantyh, 1983; Cameron *et al*., 1995; Odeh & Antal, 2001; Hannig & Jürgens, 2005), the PAG lacks direct connections to the cranial phonatory motoneuron pools in total (Mantyh, 1983; Thoms & Jürgens, 1987; Rye *et al*., 1988; Holstege, 1989); also its neural activity does not reflect frequency modulations (Larson, 1991; Düsterhöft *et al*., 2004). Furthermore, destruction of the PAG does not abolish species-specific vocalization electrically elicited from the pons (Siebert & Jürgens, 2003). Finally, a recent study showed that the PAG is also active during human speech (Schulz *et al*., 2005). This points to a more general role of the PAG in vocal behaviour, probably having to do more with the gating of vocal behaviour than with vocal motor coordination.

We show here that the motor pattern generator for frequency-modulated vocalizations is located in the VLPB. The present study leaves open the question of where non-frequency-modulated call types are coordinated. This might happen in the reticular formation caudal to VOC, as this region also has connections to all cranial phonatory motoneuron pools (Thoms & Jürgens, 1987; Cunningham & Sawchenko, 2000). Therefore, besides the pattern generation of mastication and respiration, the pontine and medullary brainstem also contains the pattern generator for vocalization.

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Abbreviations

MA, group of neurons showing neuronal activity to vocalization and to mastication; PAG, periaqueductal grey; VLPB, ventrolateral pontine brainstem; VOC, group of neurons, showing neuronal activity exclusively to vocalization.

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