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Audio–vocal interaction in the pontine brainstem during self-initiated vocalization in the squirrel monkey

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Abstract

The adjustment of the voice by auditory input happens at several brain levels. The caudal pontine brainstem, though rarely investigated, is one candidate area for such audio–vocal integration. We recorded neuronal activity in this area in awake, behaving squirrel monkeys (*Saimiri sciureus*) during vocal communication, using telemetric single-unit recording techniques. We found audio–vocal neurons at locations not described before, namely in the periolivary region of the superior olivary complex and the adjacent pontine reticular formation. They showed various responses to external sounds (noise bursts) and activity increases (excitation) or decreases (inhibition) to self-produced vocalizations, starting prior to vocal onset and continuing through vocalizations. In most of them, the responses to noise bursts and self-produced vocalizations were similar, with the only difference that neuronal activity started prior to vocal onset. About one-third responded phasically to noise bursts, independent of whether they increased or decreased their activity to vocalization. The activity of most audio–vocal neurons correlated with basic acoustic features of the vocalization, such as call duration and/or syllable structure. Auditory neurons near audio–vocal neurons showed significantly more frequent phasic response patterns than those in areas without audio–vocal activity. Based on these findings, we propose that audio–vocal neurons showing similar activity to external acoustical stimuli and vocalization play a role in olivocochlear regulation. Specifically, audio–vocal neurons with a phasic response to external auditory stimuli are candidates for the mediation of basal audio–vocal reflexes such as the Lombard reflex. Thus, our findings suggest that complex audio–vocal integration mechanisms exist in the ventrolateral pontine brainstem.

Introduction

The auditory system and the vocal control system do not function independently of each other. On the one hand, vocal output is directly influenced by auditory feedback. An example is the 'Lombard' reflex in humans, which is characterized by an increase in vocal intensity when the auditory feedback of the speaker's own voice is masked by noise (Lombard, 1911). This reflex is also found in other primates and in species, such as the cat, with innately determined acoustical structures in their vocalizations (Sinnott *et al*., 1975; Nonaka *et al*., 1997; Brumm *et al*., 2004). On the other hand, auditory perception is directly influenced by the vocal output. An example is the middle-ear reflex, in which the auditory input is attenuated by contraction of the middle ear muscles during self-produced sounds in order to protect the inner ear (Carmel & Starr, 1963; Salomon & Starr, 1963; Suga & Jen, 1975). Damping of inner ear activation during one's own vocalizations is also achieved via the action of the olivocochlear system (OCS; Warr, 1992; Goldberg & Henson, 1998).

In order to adjust a speaker's voice to the conditions of the acoustical environment, i.e. tune the auditory sensitivity to sounds of possible importance from the environment and, at the same time, protect the inner ear during self-produced vocalization, complex audio–vocal integration mechanisms exist at almost all levels of the primate brain. Single-unit recording studies in the monkey and bat have revealed audio–vocal interactions in the auditory cortex, medial geniculate body, inferior colliculus and paralemniscal area at the midbrain–pons transition (Müller-Preuss, 1979; Schuller, 1979; Müller-Preuss & Ploog, 1981; Suga & Yajima, 1988; Metzner, 1989, 1993; Eliades & Wang, 2003; Tammer *et al*., 2004). All these structures contain neurons that are activated by vocalizations played back from a loudspeaker, but are inhibited to a certain degree by the same vocalizations if self-produced. Vocalization-induced attenuation of neural responsiveness has also been described in the bat ventral nucleus of the lateral lemniscus (Suga & Schlegel, 1972; Suga & Shimozawa, 1974). Further centres of audio–vocal integration in the more caudal brainstem have not been characterized yet. The superior olivary complex (SOC), including the periolivary region (POR), is a candidate area, as auditory input is present (e.g. Helfert & Aschoff, 1997) and vocalization output is blocked by injection of kynurenic acid (a glutamate antagonist) into this area (Jürgens, 2000). Hence, we explored the SOC and surrounding areas with microelectrodes for neurons showing both auditory- and vocalization-related responses. We used a telemetric single-neuron recording technique, which allowed recording of sound- and vocalization-related activity in freely moving animals (Grohrock *et al*., 1997; Jürgens & Hage, 2006).

In the POR and the adjacent pontine reticular formation, we found neurons with properties consistent with audio–vocal integrators. They responded to acoustic stimuli and were either inhibited or excited by the act of self-produced vocalization. The contribution of these neurons to audio–vocal regulation processes is discussed.

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Materials and methods

Animals and anaesthesia

The 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 repeated injections of ketamine and xylazine (half and quarter initial dosages).

Surgery and implantation of platform and electrodes

The anaesthetized animals were placed in a stereotaxic apparatus. The surgery was performed under aseptic conditions. The skin over the frontal and parietal parts of the skull was removed and a platform implanted with the aid of four stainless steel screws, anchored in the skull with nuts and embedded in dental acrylic (Paladur; Kulzer, Wehrheim, Germany). The platform consisted of a 30 × 30 × 4 mm acrylic plate with an embedded grid of 12 × 12 stainless steel guiding tubes (outer diameter 0.81 mm, inner diameter 0.52 mm, centre-to-centre distance 0.81 mm orthogonally, 1.13 mm diagonally) covering the stereotaxic coordinates of the brainstem according to the brain atlas of Emmers & Akert (1963). After fixation of the platform, the wound edges were adapted to the head mount and the skin incisions rostral and caudal to the mount were sutured. Post-operative care consisted of analgesia with buprenorphine (0.003 mg/kg body weight) and antibiotic treatment. After 4 weeks of recovery, the animals were implanted with pairs of 80-μm-diameter quartz-insulated platinum–tungsten microelectrodes (impedance 1–2 MΩ; Thomas Recording, Marburg, Germany) under general anaesthesia (30 mg ketamine, 6 mg xylazine and 0.06 mg atropine sulphate in 0.6 mL sterile water per kg body weight). The electrodes were inserted into the brain with the aid of a custom-made ultra-light microdrive fixed on the platform (Jürgens & Hage, 2006). They were running within stainless steel stabilizing tubes which themselves ran within another pair of guiding tubes (outer diameter 0.47 mm, inner diameter 0.25 mm). The guiding tubes served as indifferent electrodes and ended just above the structures to be explored. The distance between the two electrodes of a pair was 1.13 mm. Finally, the electrodes were connected to the transmitters (see below), and a protection cap (Plexiglas) was placed on the platform.

Telemetry

The neuronal activity picked up by the electrodes was amplified by a MOSFET preamplifier and fed into a custom-made transmitter circuit. The circuit was completely built with surface-mounted technology components and assembled on a single 20 × 28 mm printed circuit board. Transmitting coils and radiofrequency stages were mounted on one side of the circuit board, while the other side held the preamplifier and 3-V lithium battery (for details of the electronic circuitry and telemetry setup, see Grohrock *et al.*, 1997). One of the two transmitters mounted on the platform had two channels. The first channel was used to record the neuronal activity and the second transmitted the signal of a piezo-ceramic skull vibration sensor. This sensor picked up vibrations of the skull due to vocalizations of the experimental animal. Self-produced vocalizations could thus be distinguished from other sounds, especially from vocalizations of the other animals of the group, by comparing the signals coming from the room microphone and the skull vibration sensor. Carrier frequencies of the transmitters were between 100 and 150 MHz. The whole headstage, including platform, microdrive, amplifiers, transmitters and protection cap, had a weight of 32 g (Jürgens & Hage, 2006). The recording sessions took place in a cage of 2.4 m height × 0.8 m length × 0.8 m width, in which the experimental animal was housed with one or two other animals. Two additional groups of three squirrel monkeys each were held in separate cages in the same room with visual, acoustic and restricted tactile contact with the experimental animal. The room was lined with foam mats to reduce echos.

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

Recording procedure

Recording sessions lasted 10–15 min and were held twice a day during feeding time, as the occurrence rate of vocalizations was the highest during that time. Before each session, the experimental animal was caught and placed in a monkey chair. There, the electrodes were moved into a new position and batteries were exchanged, if necessary. The distance between successive recording positions was 50 or 100 μm depending on the recording site. After the electrodes had been advanced to the new positions, the animal was brought to the observation cage and recording started. At the end of an electrode track, the electrodes were removed together with their guiding tubes, and a new pair of electrodes was implanted at a new position.

Neuronal activity was recorded during all call types uttered. Quantitative data analysis was performed for a highly frequency-modulated type of vocalization (trill) with a specific repetitive character (Fig. 1). Trill vocalizations were uttered in a sufficient number ($n = 6$) for a quantitative and statistical analysis of the recordings at any of the electrode positions in the brain. Low-pitched nonrhythmic caw vocalizations (Fig. 1) were uttered only at a few

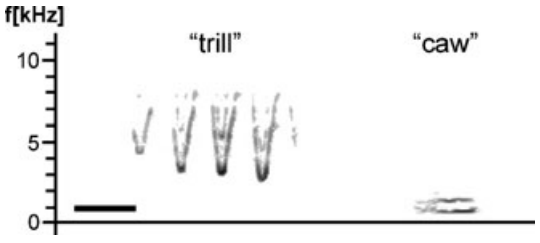

FIG. 1. Example of a spontaneously uttered trill and caw vocalization of the squirrel monkey. Sonograms as recorded with the bone vibration sensor. Intensity is represented by grey level. *X*-axis, time. Scale bar, 100 ms.

positions. Hence, neural responses to them were not quantitatively analysed. To test whether the recorded neurons showed a consistent auditory response, bursts of white noise were used as acoustic stimuli in addition to the animal's own vocalization and vocalizations from its group mates. Broadband noise [20 Hz–200 kHz, 80 dB sound pressure level (SPL)] was produced by a generator (Sine/Noise Generator Type 1049; Brüel & Kjaer, Quickborn, Germany) and shaped by an electronic switch (Uni-Ulm-Elektronik, Ulm, Germany) into 300-ms bursts (including 10-ms rise and fall times) with 700-ms intertone intervals. The output signal of the generator was fed through a power amplifier (TA-F335R; Sony, Köln, Germany) to a dynamic speaker (Mariner 300; Mediacraft, Frankfurt, Germany) located in the animal room. The speaker covered frequencies between 65 Hz and 30 kHz and had a flat ±3 dB response in a range of 1–20 kHz at the site of the animal's ear. Bursts were presented while the experimental animal was sitting quietly in a distance of 60–120 cm from the speaker. The SPL of the white noise bursts were measured at a distance of 90 cm from the speaker (6.5-mm calibrated microphone 4135 and measuring amplifier 2231; Brüel & Kjaer). White noise was used as almost all auditory neurons in the pontine brainstem show responses to this kind of stimulus (e.g. Tsuchitani & Boudreau, 1966; Tsuchitani, 1977; Caird & Klinke, 1983; Irvine & Jackson, 1983).

Data analysis was carried out using the software Spike2, Version 5 (CED). For the identification of vocalization-correlated and auditory units, conventional peri-event time histograms (PETH) and peri-stimulus time histograms (PSTH), respectively, were constructed after the original recording had run through a spike-sorting procedure (template-based spike clustering). Figure 2 shows an example of separating three spike forms from the original recording at a given electrode position. 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. Fibre recordings were distinguished from those of neurons by spike shape and amplitude (compare Fig. 2). Fibre recordings can practically be excluded because of the low-impedance electrodes.

Histology

At the end of the experiments, the animals were killed with an overdose of pentobarbital sodium. They underwent perfusion with warm physiological saline, followed by 4% paraformaldehyde. The brains were removed and, after a postfixation time of 1 week in 4% paraformaldehyde followed by 20% sucrose for cryoprotection, were cut on a cryotome at 40 µm in the frontal plane. The sections were stained with Cresyl Violet. Every second section was counterstained immunohistologically against glial fibrillary acidic protein according to Benevento & McCleary, 1992). Histological evaluation (Fig. 3) was made using the stereotaxic atlas of Emmers & Akert (1963).

The experiments were approved by the animals ethics committee of the district government Braunschweig, Lower Saxony, Germany. The experiments were performed according to the principles regarding the care and use of animals adopted by the American Physiology Society, the Society for Neuroscience, and the specifications of the German Animal Welfare Law for the prevention of cruelty to animals. Care was taken to minimize the number of animals used. All three experimental animals behaved normally to human caretakers and to other animals in the colony.

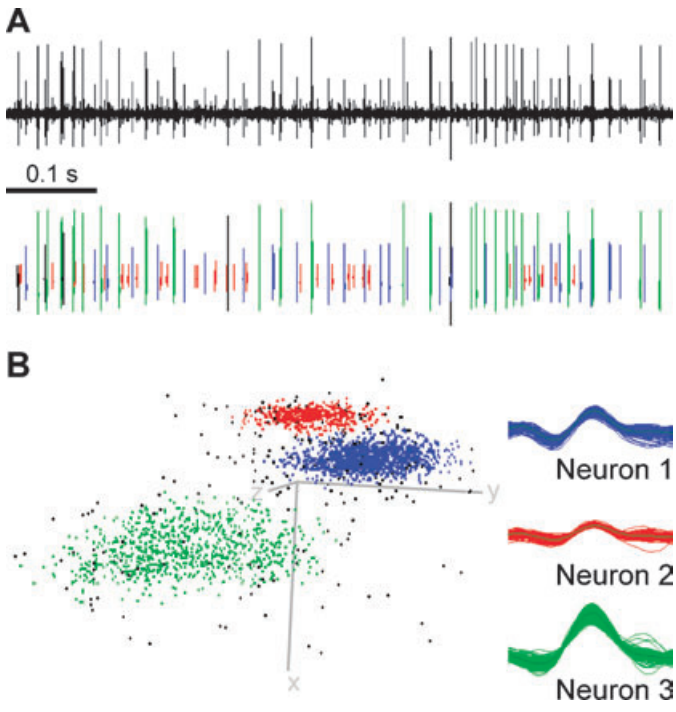

FIG. 2. Example of a spike-sorting procedure with three telemetrically recorded neurons. (A) Neuronal recording at a given electrode position before (upper trace) and after (lower trace) separating three spike forms with spike sorting. (B) Cluster plots identifying three neurons shown in 3-D space based on the principal component analysis of Spike2, Version 5 (CED, Cambridge, UK). The appropriate spike waveforms are superimposed for each neuron on the right side, underscoring a proper spike sorting procedure.

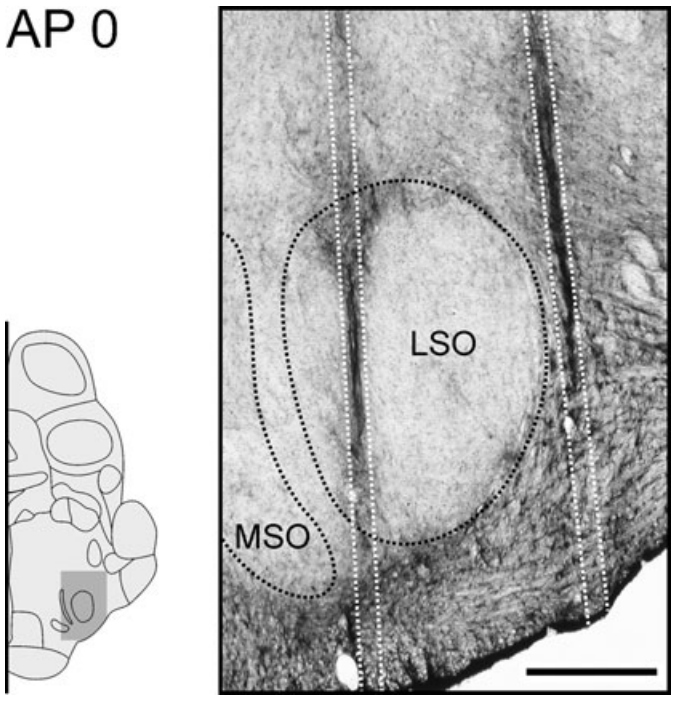

FIG. 3. Frontal sections through the ventrolateral pontine brainstem of one of the experimental animals. Left: complete hemisection at the midbrain–pons transition at the stereotaxic coordinate AP 0 from Emmers & Akert (1963). Right: glial fibrillary acidic protein staining of the brain area indicated in grey on the left. Two tracks of electrodes (white dashed lines) are visible. The electrodes had been removed several months before killing the animal. MSO, medial nucleus of the SOC; LSO, lateral nucleus of the SOC (modified from Jürgens & Hage, 2006). Scale bar, 500 µm.

Results

A total of 714 positions were explored in the ventrolateral pontine brainstem of three squirrel monkeys, *Saimiri sciureus*. Single neurons were isolated at 453 positions. Neurons were located in the SOC (195 units), the ventral nucleus of the lateral lemniscus (vLL; 19 units), the lateral lemniscus (LL; 16 units) and the adjacent pontine reticular formation (FRP; 223 units). The recorded neurons could be divided into three major types, based on their responses to spontaneously uttered, self-produced vocalizations and other acoustic stimuli. The first type, called auditory neurons, responded to noise bursts and showed no activity prior to the onset of own vocalizations (295 units); 146 neurons of this type were observed in the SOC, 19 in the vLL, 16 in the LL and 114 in the adjacent FRP. The second type, nonauditory neurons, were spontaneously active but could not be driven by acoustic stimuli or the animal's own vocalizations. This type of neurons is not subject of this article and will not be considered further here. The third type, called audio–vocal neurons (AVU; 27 units) are the main focus of this study. Sixteen neurons of this type were recorded in the SOC and 11 in the FRP adjacent to the SOC and vLL. AVU were either inhibited or excited by the animal's self-produced vocalizations, with a change of neuronal activity starting prior to vocal onset. In addition, they were driven by the noise bursts and vocalizations of group mates.

Audio–vocal units (AVU)

Quantitative analysis of AVU was conducted exclusively with responses to trill vocalizations, as trills were uttered at all positions at which AVU could be isolated (median number of trill calls per position, 15.6 ± 10.5; $n = 27$ positions). Based on their vocalization-related activity, AVU were subdivided into two distinct types: most (18 of 27) of the recorded AVU showed an increase in activity immediately before and during self-produced vocalization (excited AVU or EAVU). The remaining AVU (9 of 27) were characterized by a suppression of spontaneous activity prior to and during self-produced vocalization (inhibited AVU or IAVU). Examples are shown in Fig. 4.

The locations of AVU in the brain are shown in Fig. 5. Most (19 of 27) AVU were found in the POR of the SOC outside the three main nuclei, the medial and lateral superior olive (MSO and LSO) and the medial trapezoid body (MTB). The AVU were located mediodorsal to the MSO (6/19), dorsal to the LSO (5/19), in between MSO and LSO (2/19) and in the caudal POR (6/19). A smaller group of AVU (8 of 27) was located in the FRP dorsal and caudal to vLL. The locations of excited and inhibited AVU showed little overlap. Almost all EAVU were positioned more laterally in the POR and FRP than were the IAVU (Fig. 5B).

Vocalization-correlated excitation or inhibition of AVU started 40–90 ms prior to vocal onset in most cases, as shown in Fig. 6. The median latency was 70 ms. There were no significant differences in latency between EAVU and IAVU (*U*-test, $P > 0.1$) nor between AVU in the SOC and FRP (*U*-test, $P > 0.1$).

Some AVU showed vocalization-correlated activity which reflected specific acoustic features of the vocalizations.

Syllable correlation

In 10 of 27 AVU, activity changed in the rhythm of the syllable structure of the trill vocalizations (Fig. 5C, solid symbols). A syllable-correlated activity is shown in Fig. 9B in a PETH. As trill syllables were constant in their duration (median time between the minima of two consecutive syllables was 71.9 ± 2.6 ms), syllable-correlated activity was determined by the detection of syllable-synchronized discharges in PETHs (Fig. 9B). Different call durations were mainly due to different numbers of syllables in trill calls.

Duration correlation

In 10 of the 19 AVU that could be tested for this feature, a significant correlation ($P < 0.05$) between the duration of a vocalization and the duration of the corresponding neuronal activity was found (Fig. 5C). Neurons showing call duration-correlated neuronal activity had

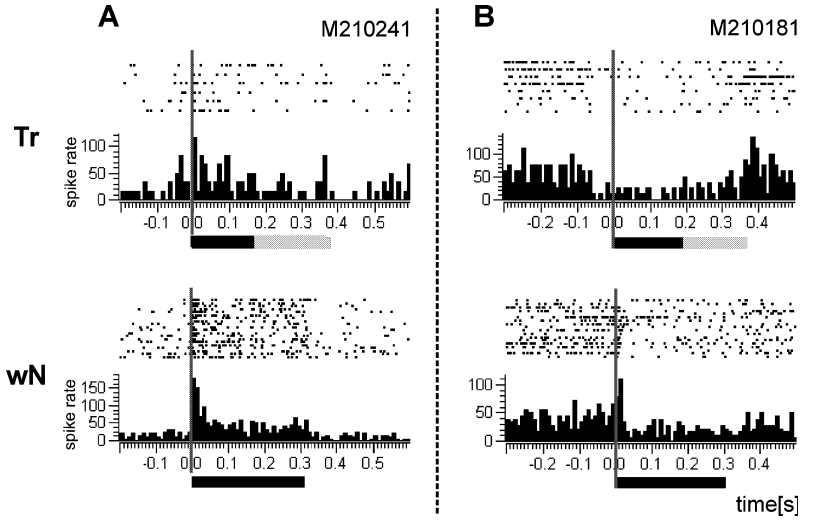

FIG. 4. (A and B) Activity of two audio–vocal neurons. Activity during self-produced trill vocalizations (Tr) and white noise bursts (wN). Each panel shows as raster displays (top) rows of dots, each representing series of action potentials during self-produced trills or bursts of noise, and the summation of this activity in PETHs (bottom). Black bars below the trill-related activity indicate the onset of all trill vocalizations (time 0.0) and the duration of the shortest trill emitted; the grey bars indicate the duration of the longest trill. Different call durations are mainly due to different numbers of syllables in trill vocalizations. Black bars below the noise-related activity indicate the onset and duration of the noise bursts (300 ms). (A) EAVU with activity starting ~40 ms prior to vocal onset. The neuron shows a phasic–tonic noise response to noise bursts. (B) IAVU with a reduction in spontaneous activity just before and during vocalization. A phasic excitation followed by a tonic inhibition of spontaneous activity occurs to noise bursts. Bin size, 5 ms.

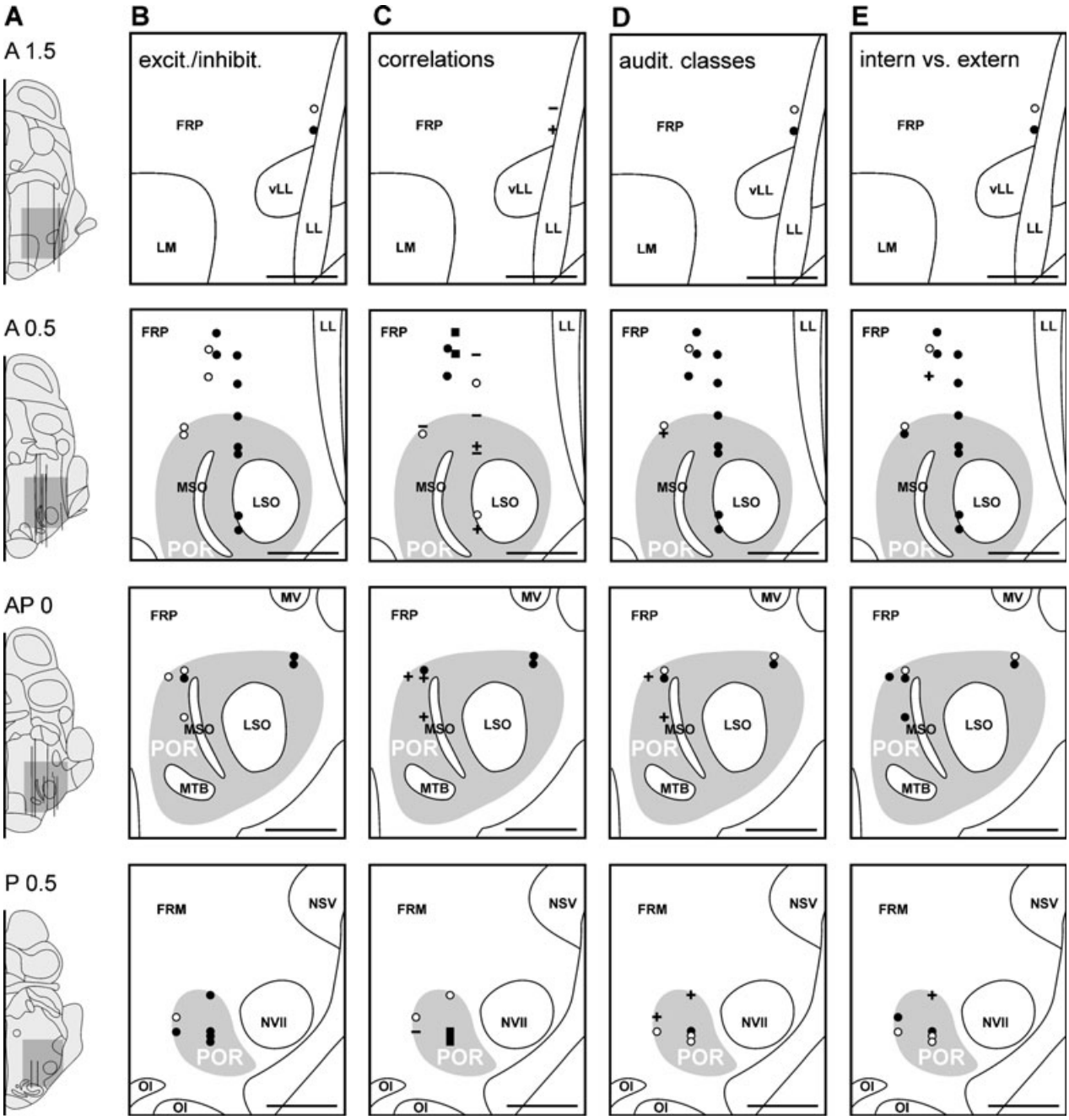

FIG. 5. Frontal views of the squirrel monkey's brainstem. (A) Hemisections at the midbrain–pons transition (stereotaxic coordinates from Emmers & Akert, 1963) with vertical lines indicating the extent of the explored electrode tracks. Electrode tracks originally positioned on the left side were mirrored to the right for better overview. Highlighted squares indicate the ventrolateral pontine brainstem shown enlarged in B–E. (B) Positions of the recorded AVU. Neurons showing a vocalization-correlated excitation in their activity (EAVU) are indicated by ● neurons showing an inhibition (IAVU) are indicated by ○. (C) Positions of the AVU classified according to their activity patterns during trill vocalizations: call duration- and syllable-correlated activity (■), syllable-correlated activity only (●), call duration-correlated activity only (○), no correlated activity (+), specific correlations could not be tested (-). (D) Auditory response patterns of the AVU: Tonic (●), phasic (○) and inhibited (+). (E) Distribution of AVU showing similar activity to self-produced vocalization and white noise bursts (●), phasic activity (○) or opposite activity patterns (+). FRM, medullary reticular formation; FRP, pontine reticular formation; LL, lateral lemniscus; LM, medial lemniscus; LSO, lateral nucleus of the SOC; MSO, medial nucleus of the SOC; MTB, medial trapezoid body; MV, motor trigeminal nucleus; NSV, spinal trigeminal nucleus; NVII, facial nucleus; OI, inferior olive; POR, periolivary region; vLL, ventral nucleus of the lateral lemniscus. Scale bars, 500 μm.

r-values between 0.63 and 0.99 (median *r*-value, 0.88). The duration correlation is specified for five neurons in Fig. 7.

Five neurons showed both syllable- and duration-correlated activity (Fig. 5C).

AVU responded in different ways to acoustic stimuli. Based on PSTHs, we subdivided the neuronal response characteristics to the noise bursts into three distinct types (Fig. 8). (i) Tonic response: about half of the neurons (14/27) showed a tonic or phasic–tonic discharge pattern with short latency (< 10 ms). (ii) Phasic response: one-third of AVU (8/27) responded with a short phasic response of short latency (< 10 ms), which was sometimes followed by a weak inhibition during stimulus duration. (iii) Inhibited response: this was

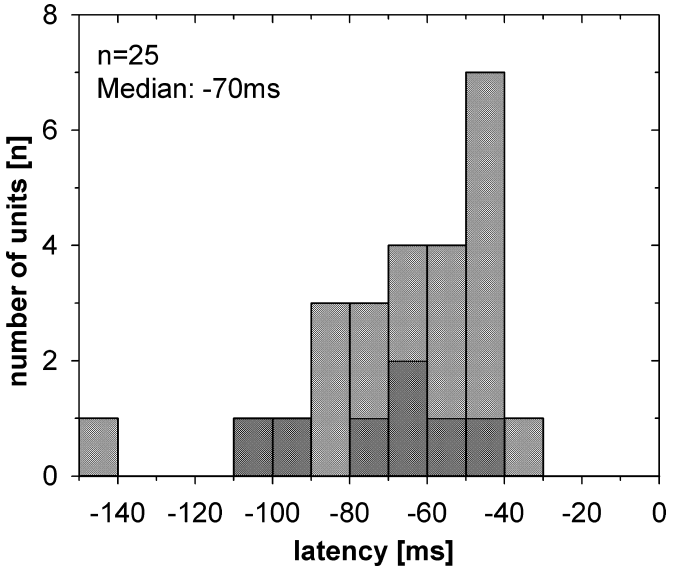

FIG. 6. Histogram of preonset times related to the beginning of vocalization for 25 out of 27 neurons recorded. IAVU, dark grey; EAVU, light grey. For two IAVU neurons preonset times could not be calculated due to low spontaneous activity. The distribution has a median of −70 ms. Bin size, 10 ms.

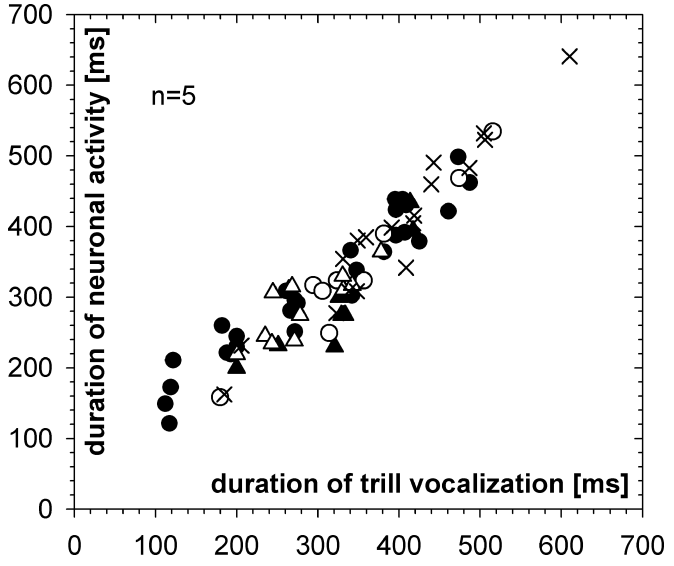

FIG. 7. Five examples of AVU, showing an activity significantly correlated with the duration of vocalizations. Different symbols represent different neurons. Each symbol stands for one series of trill vocalizations. Different durations are mainly due to different numbers of syllables in a series (Pearson's correlation, $P < 0.01$ for each single neuron shown, r-values between 0.83 and 0.98, median r-value of 0.91).

the rarest response type (5/27). After a weak phasic response at stimulus onset, neuronal activity was suppressed below the spontaneous level during the presence of the noise. Sometimes a short excitation followed after the end of the stimulus. The spatial distribution of the auditory response types of AVU are shown in Fig. 5D.

A comparison of the auditory response types of neurons with their response types to self-produced vocalizations (EAVU and IAVU) showed a significant nonhomogeneous distribution (Fisher's exact-test, $P < 0.05$). Most neurons with excitatory responses to self-produced vocalizations (EAVU) had a tonic response pattern to noise bursts (13/18). Tonic activity in IAVU, in contrast, was very rare (1/9). IAVU mainly showed phasic (4/9) or inhibited (4/9) responses. In other words, most AVU (17/27) responded similarly to external and self-produced acoustic stimuli with the only difference being that the change in neural activity started before the acoustic stimulus when self-produced (Fig. 5E). In Fig. 9A, an IAVU shows inhibition during the presence of trill vocalizations and white noise. Additionally, the neuron shows a clear off-response to self-produced as well as to external acoustical stimuli. An IAVU with a phasic response to external acoustic stimuli has already been shown in Fig. 4B. An EAVU with a syllable-related phasic–tonic activity to a series of self-produced trills and a phasic–tonic activity during the presence of noise bursts is shown in Fig. 9B.

Auditory units

Auditory neurons responded in various ways to bursts of white noise (Fig. 10). They were classified by their PSTH into eight types. By far the most neurons (183; 62%) showed a phasic–tonic response. Other response types were phasic (40 units), tonic (23 units), phasic with a weak tonic component (30 units), phasic–tonic only to part of the noise duration (eight units), double-phasic (three units), long latency (latencies >10 ms; six units), and off (two units). The spatial distribution of auditory neurons is shown in Fig. 11.

For better comparison of the response types of the auditory neurons with those of the AVU, we grouped the eight response types into three classes as shown in Fig. 10. Most neurons (206 of 295) showed a sustained activity (phasic–tonic, tonic). They were grouped together in the class 'tonic'. All types with a phasic characteristic (phasic, phasic with weak tonic activity, phasic–tonic of short duration, douple-phasic) were grouped into the class 'phasic' (81 of 295 units). Finally, the complex response types (long latency, off-response), were grouped together in the class 'complex' (8 of 295 units). In Fig. 12, the percentages of neurons in tonic and phasic response classes are shown with respect to their positions in the brain. All auditory neurons showing complex response patterns were recorded in the SOC and were left out of the statistical analysis due to small sample size. The distribution of tonic and phasic response classes in the FRP is

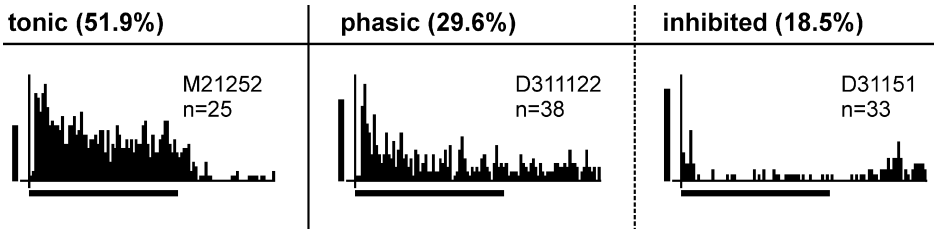

FIG. 8. Three examples of PSTHs of the response to noise bursts (80 dB SPL, 300 ms) found in AVU. Tonically responding neurons were most common (51.9%), followed by phasic (29.6%) and inhibited ones (18.5%). Numbers (n) of acoustic stimuli underlying each PSTH are given for each neuron separately. Bin size, 5 ms. Scale bars, abscissa 300 ms, ordinate 100 spikes.

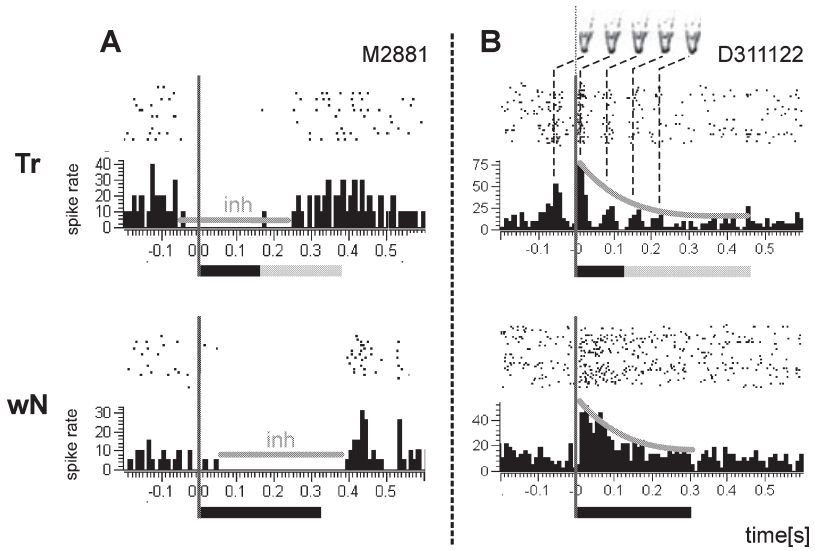

FIG. 9. PETHs of AVU showing similar activity patterns to self-produced vocalizations and bursts of white noise. (A) IAVU: the neuron shows a decrease in neuronal activity (inh) starting prior to vocal onset. The same response type is shown to noise bursts, with inhibition starting, however, after stimulus onset. (B) EAVU: A syllable-correlated activity is found, starting prior to vocal onset. The relationship between neuronal activity and trill syllables of a representative trill call are shown. The overall phasic–tonic activity (grey line) is also seen in the response to noise bursts. For further explanation, see legend to Fig. 4.

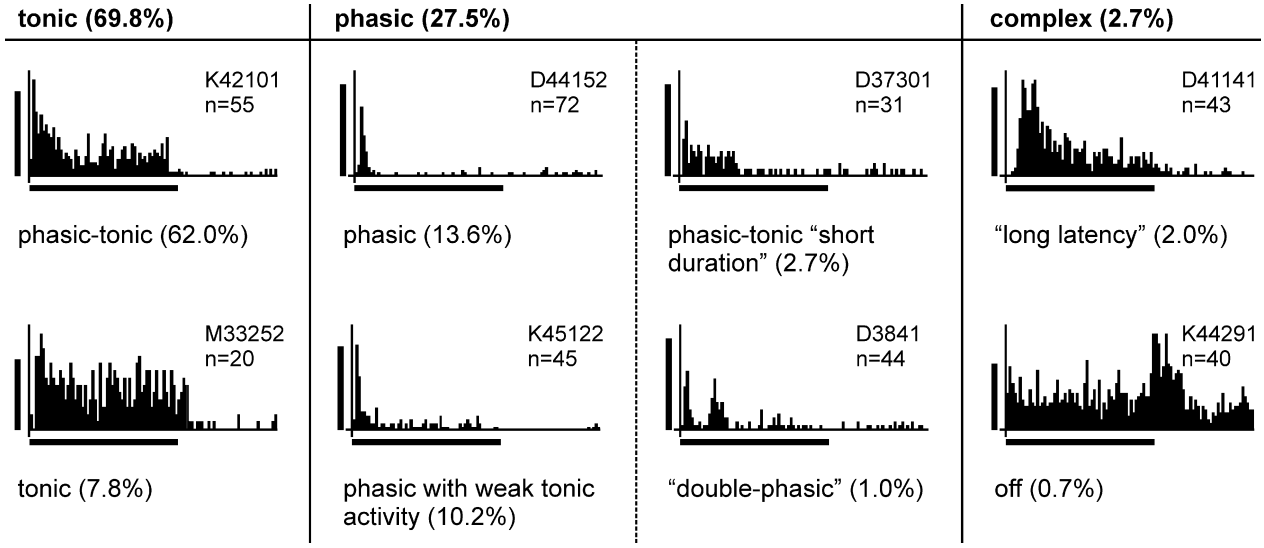

FIG. 10. Examples of PSTHs found in purely auditory neurons in response to noise bursts (80 dB SPL, 300 ms). The noise response types were grouped into three main types (tonic, phasic and complex), the percentages of which are indicated. For further explanation, see legend to Fig. 8.

significantly different from those in the SOC and the vLL/LL (χ^2-test, $P < 0.01$ in both cases): neurons with tonic and phasic responses occurred at similar rates in FRP; tonic responses dominated phasic responses in the SOC and the vLL/LL.

Response types in clusters of AVU and auditory units

In order to better understand functional relationships between AVU and auditory neurons, the auditory neurons were divided into those lying closely to AVU and others lying in areas lacking AVU. Thus 151 of 295 auditory neurons were assigned to 'AVU areas' and 144 were assigned to 'purely auditory areas'. AVU areas and purely auditory areas are indicated in Fig. 11. The distributions of auditory neurons with regard to tonic and phasic response types are significantly different between the 'AVU areas' and the 'purely auditory areas', both if all auditory neurons and if only those in the SOC are considered (χ^2-test, $P < 0.05$ and $P < 0.01$, respectively). This is shown in Fig. 13A and B. In summary, this means that significantly more auditory neurons in the 'AVU areas' have phasic responses to noise bursts and, thus, code only the beginning instead of the total duration of an external auditory stimulus, compared to auditory neurons in purely auditory areas, in which phasic responses are rare and tonic responses dominate. It is evident, however, from Fig. 11 that 22 of the 27 AVU responded in the same way (tonically or phasically) as the purely auditory neuron next to a given AVU. This indicates with high significance ($P < 0.005$, sign test) that the type of auditory response of most neurons reported here is determined by the location in the brain, irrespective of any additional auditory–vocal responsiveness.

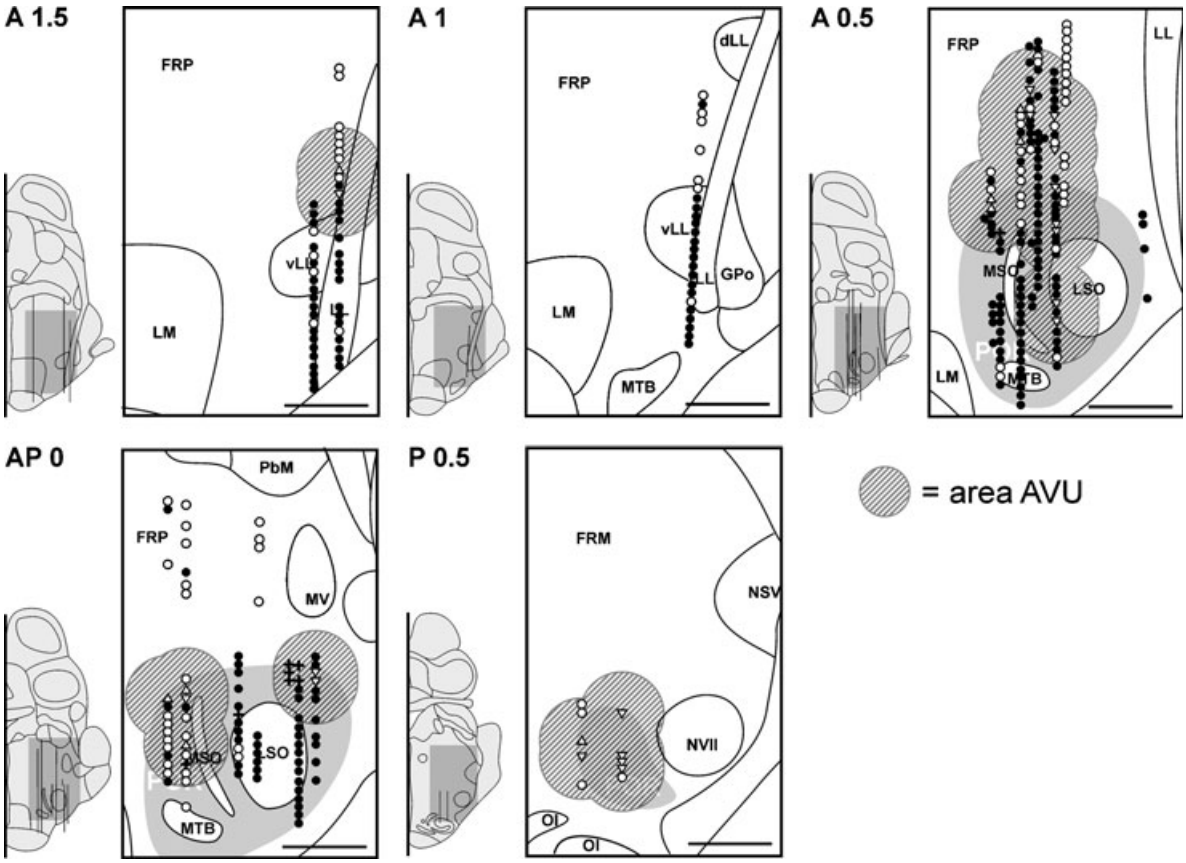

FIG. 11. Frontal views of the squirrel monkey's brainstem showing the spatial distribution of the recorded purely auditory neurons (stereotaxic coordinates from Emmers & Akert, 1963). Neurons with tonic responses to noise bursts are indicated by ●, those with phasic responses by ○, and those with complex responses by +. For comparison, the locations of IAVU are added as △ and of EAVU as ▽. Additionally, hatched areas mark regions close to AVU (circles with 250-μm radius) dividing auditory neurons into two different groups: neurons lying close to AVU ('area AVU') and those in purely auditory areas. For further explanation, see legend to Fig. 5. Scale bars, 500 μM.

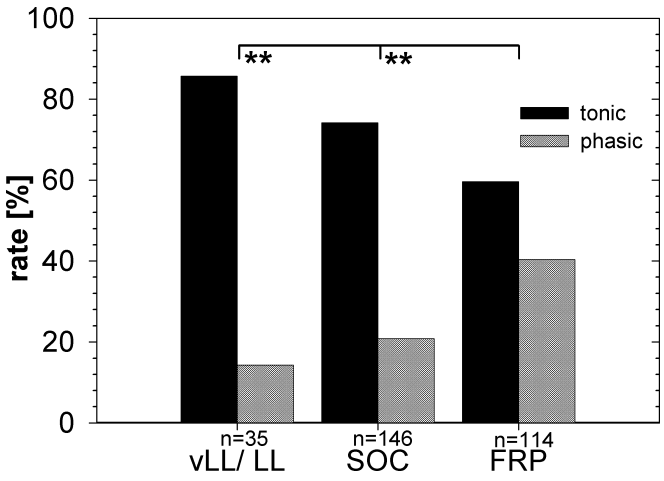

FIG. 12. Percentages of the three main types of noise response patterns of auditory neurons in different brain areas (vLL/LL, SOC and FRP). Statistically significant differences between the brain structures are indicated by asterisks (χ^2, $^{**}P < 0.01$). Complex response patterns were left out of statistical analysis due to small sample size.

When we compared auditory responses of EAVU and IAVU with those of adjacent purely auditory neurons in the same electrode tract (Fig. 11), we found a further statistically significant relationship ($P < 0.01$, Fisher's exact test): except for the very caudal POR (stereotaxic coordinate P 0.5), EAVU were more frequently colocalized with tonically responding auditory neurons (16/20) while IAVU were more frequently colocalized with phasically responding auditory neurons (8/11).

Vocalization-specific AVU

At eight out of 27 positions where AVU could be isolated, experimental animals produced, besides trills, also caws, which represent low-pitched nonrhythmic sounds without marked frequency modulations (median number of caw vocalizations per position, 11.5 ± 10.6). These positions were found dorsomedial to the MSO and in the caudal part of the POR. Five of these AVU did not change their neuronal activity to self-produced caws, but did so to trills. Only three AVU showed vocalization-correlated activity during self-produced trills and caws.

Discussion

The present study has shown the following. (i) AVU are present at brainstem locations that have not been described as audio–vocal before: the superior olivary complex and the caudal pontine reticular formation. (ii) These AVU divided up into two groups, those being excited (EAVU) and those being inhibited (IAVU) prior to and during

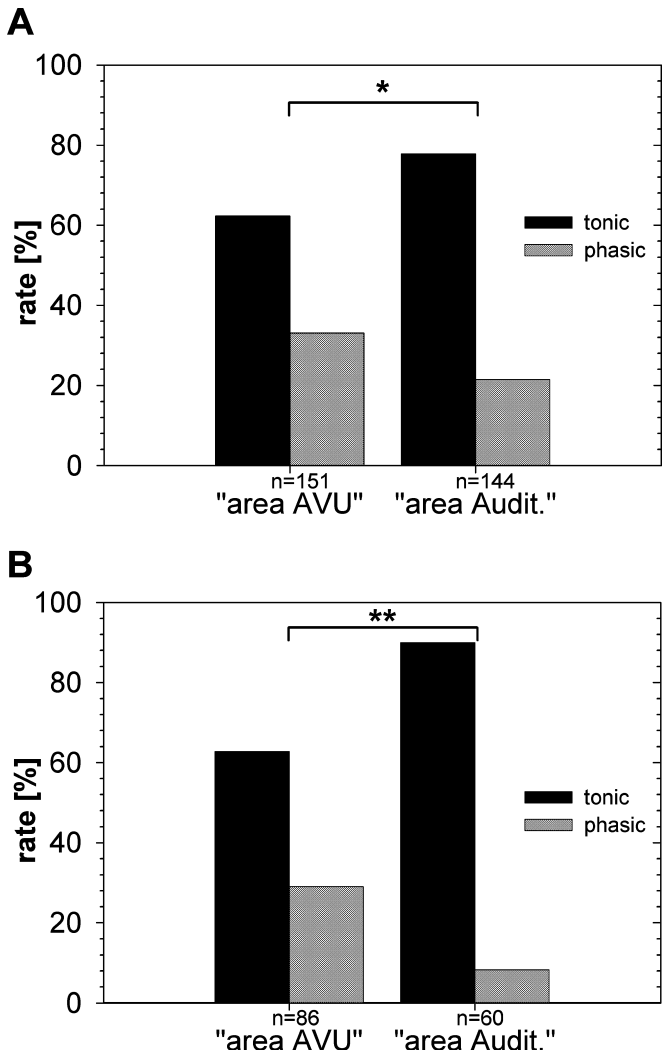

FIG. 13. Percentages of the three main types of noise response pattern in brain areas containing AVU ('area AVU') and brain areas containing only auditory units ('area Audit.') for (A) all auditory neurons and (B) auditory neurons of the SOC. Statistically significant differences between the distributions are indicated by asterisks (χ^2, $^*P < 0.05$, $^{**}P < 0.01$). Complex response patterns were left out of statistical analysis due to small sample size.

self-produced vocalization. Populations of EAVU and IAVU were largely separated in the brainstem, with EAVU being located more laterally than IAVU. (iii) Most AVU responded to external auditory stimuli in the same way as to self-produced vocalizations. (iv) Populations of auditory neurons lying close to AVU showed distributions of tonic and phasic noise response patterns different from those in purely auditory areas. (v) Except for the very caudal periolivary region, most EAVU were colocalized with tonically responding and most IAVU with phasically responding purely auditory neurons.

Audio–vocal activity in the caudal pontine brainstem

In the present study, 2/3 of AVU showed an increase in neuronal activity to self-produced vocalizations (EAVU). This is in contrast to other studies dealing with AVU, where most neurons showed a vocalization-correlated decrease in neuronal activity. In the auditory cortex of monkeys, three out of four AVU showed a vocalization-correlated inhibition (IAVU; Eliades & Wang, 2003); in the inferior colliculus all AVU behaved in this way (Müller-Preuss, 1989; Tammer *et al*., 2004). Inhibition of sound-evoked potentials during emission of echolocation sounds was also demonstrated at the level of nuclei of the lateral lemniscus (midbrain–pons transition) in bats. In two pioneering studies, Suga & Schlegel (1972) and Suga & Shimozawa (1974) demonstrated that vocalization influenced auditory processing in mammals. They found that evoked potentials at the level of the nuclei of LL were attenuated during emission of an orientation call compared to passive acoustical stimulation.

The only studies dealing with audio–vocal activity on the single-cell level in the brainstem were performed by Metzner (1989, 1993). In this case, recordings were carried out in the paralemniscal area (PLA) at the midbrain–pons transition (the locations correspond to rostro-caudal coordinates of A > 0.5 in the monkey; compare Fig. 5). These neurons had activity characteristics of EAVU (20%), IAVU (50%) and of a kind not found in the present study (30%). Beyond these differences in proportions of EAVU and IAVU between Metzner's and our present work, differences in responses of IAVU to auditory stimuli exist. In the bat, IAVU responded tonically to sounds (Metzner, 1993) while in the present study most IAVU showed a phasic response. Additionally, purely auditory neurons showed different response patterns in the PLA of the Metzner studies compared to FRP in the present study. While three out of four auditory neurons in the PLA responded in a manner similar to auditory neurons in the nuclei of LL, significantly different response patterns were found between FRP and vLL in the present study. The distributions and occurrence rates of auditory response patterns in our present study was in agreement, however, with those of other studies of auditory responsiveness (SOC: Tsuchitani, 1982; Harnischfeger *et al*., 1985; Behrend *et al*., 2002; vLL: Aitkin *et al*., 1970; Metzner & Radtke-Schuller, 1987; and FRP: Irvine & Jackson, 1983). Based on these comparisons, it seems very unlikely that AVU in the FRP of the present study correspond to those reported for the PLA in the Metzner (1989 and 1993) studies.

In conclusion, our present study is the first to demonstrate audio–vocal neurons in the caudal pontine reticular formation and the superior olivary complex. Activity patterns and anatomical positions of these neurons are, on average, significantly different from AVU reported at more rostral locations in the brain.

Vocalization pathways and AVU in the pontine brainstem

AVU showed vocalization-correlated excitation or inhibition prior to vocal onset, indicating that they get input from the vocalization pathways or from AVU in the upper auditory pathways. AVU in the auditory cortex get direct input from the cingulate cortex, an important structure for vocalization initiation (Müller-Preuss *et al*., 1980; Gooler & O'Neill, 1987). Stimulation of the cingulate cortex led to inhibition of auditory neurons in the auditory cortex. A cingulate cortical input on auditory neurons of the pontine brainstem has not been demonstrated yet. Direct input from the vocalization pathway, however, might come from a recently found vocalization area in the ventrolateral pontine brainstem (Hage & Jürgens, 2006), as the activity of most neurons there was closely correlated to the syllable structure and/or the duration of trill vocalizations, properties which were also found in about half of the AVU recorded here. The other half of the AVU did not follow the rhythm of trill syllables and thus did not show an activity correlated with call patterns. For these neurons we suggest that they received vocalization-related input indirectly, possibly via projections from the auditory cortex (e.g. Mulders & Robertson, 2001) or the inferior colliculus (e.g. Huffman & Henson, 1990; Mulders & Robertson, 2002), both of which are known to modulate

pontine activity (e.g. Khalfa *et al*., 2001; Xiao & Suga, 2002; Groff & Liberman, 2003; Mulders & Robertson, 2005).

Modulation of the auditory system by vocalization

Most AVU (63%) did not discriminate in their PSTH patterns between internal and external acoustic stimuli. Except for starting their activity prior to self-produced vocalizations, they are unable to detect sound from such vocalizations against other external auditory input, as was proposed for AVU in higher auditory centres (Eliades & Wang, 2003; Tammer *et al*., 2004). The AVU described here may be involved in a general modulation of cochlear sensitivity to self-produced and external sounds. In the pontine brainstem, two systems are known to modulate cochlear sensitivity, the OCS, which has its efferent neurons mainly in the POR of the SOC, and the middle-ear reflex, which has its motoneurons ventrolateral to the trigeminal motor nucleus and ventromedial to the facial nucleus in primates (M. stapedius, Parnes *et al*., 1982; Thompson *et al*., 1985; M. tensor tympani, Rouiller *et al*., 1986; Gannon & Eden, 1987). As the motoneurons of the middle-ear muscles differ in their activity patterns from the neurons recorded here (Suga & Jen, 1975; Vacher *et al*., 1989) and are located outside the explored area, we can exclude the middle-ear reflex as a possible function represented by the AVU activity.

It seems more probable that AVU which did not differentiate internal from external acoustic stimuli are part of the OCS. Anatomical evidence indicates that neurons of the lateral OCS in the squirrel monkey cover a POR area lateral and caudal to the MSO and those of the medial OCS a POR area medial, rostral and ventral to the MSO in the same anatomically scattered manner as was seen for AVU in the present study (Guinan *et al*., 1972a,b; Thompson & Thompson, 1986; compare Fig. 5B). Furthermore, response properties of these nondifferentiating AVU were similar to responses of OCS neurons to sounds as reported by Liberman (1988) and Brown *et al*. (2003). The lateral OCS projects to the inner hair cell area in the cochlea and the medial OCS innervates the outer hair cells (Guinan *et al*., 1983, 1984; Warr, 1992). Activation of the medial OCS has a protective effect on the cochlea against overstimulation by loud sounds and leads to a suppression of cochlear output (e.g. Wiederhold & Kiang, 1970; Liberman, 1988; Patuzzi & Thompson, 1991; Reiter & Liberman, 1995), while activation of the lateral OCS can have increasing and decreasing effects on the amplitude of the cochlear output (e.g. Groff & Liberman, 2003; Mulders & Robertson, 2005). We showed in this study that the activity of most AVU was similar during own vocalizations and the perception of external sounds, with the only difference that own vocalizations were anticipated in the discharge patterns. Thus, a possible function of such an anticipation could be an efficient modulation of and, especially, protection of the cochlear processing against loud self-produced vocalizations.

Summarizing the physiological and anatomical data, we propose that most AVU serve as neurons of the OCS. If this hypothesis is confirmed by further experiments, such as single-unit recording studies analysing the auditory responses of AVU in a more specific way or by combined tracer studies demonstrating direct anatomical projections from the vocalization pathway to the OCS, this would mean that the auditory system is modulated by self-produced vocalizations at the cochlear and lower brainstem level.

Modulation of the vocal motor output from auditory site

Nearly one-third of AVU responded phasically to external sounds. In addition, auditory neurons close to AVU showed this kind of response significantly more often than in purely auditory areas (Figs 11 and 13). Thus, AVU and auditory neurons close to AVU generally have information about the onset, but not the duration, of an external auditory stimulus. AVU with phasic responses to external sounds might play a specific role in basal audio–vocal reflexes, such as the Lombard reflex, which is characterized by an increase in vocal intensity when the auditory feedback of the vocalizer's own voice is masked by noise. It can be found in monkeys and humans (Lombard, 1911; Brumm *et al*., 2004). Furthermore, deafened kittens and monkeys show an increase in vocal intensity when compared with normal-hearing animals (Romand & Ehret, 1984; Talmage-Riggs *et al*., 1972). This means that the intensity of one's own voice is automatically up-regulated if it has a less than critical level difference from the acoustic background. Earlier studies assumed that the Lombard reflex arc is located in the brainstem, because of its survival in decerebrated cats (Nonaka *et al*., 1997). With their unique properties of tonically modulating the afferent sensitivity to self-produced sounds and phasic or reversed responding to external sounds, the EAVU in the caudal and dorsal POR as well as the adjacent FRP are candidates for mediating the Lombard reflex. Their vocalization-related activity could provide the decisive difference in the cochlear responses to self-produced compared to external sounds over a large range of intensities of external sounds.

Besides the Lombard reflex, another audio–vocal effect can be found in primates. This is characterized by an acoustically induced suppression of vocal output for $\sim$100 ms after another animal has started to vocalize (Hage, 2005). This suppression points to a direct input from the auditory system into the vocal motor-output system. Again, a phasic response pattern would be sufficient for the initiation of such an effect.

All EAVU with phasic responses to external sounds show a clear correlation in their activity with specific acoustic features of own vocalizations, such as syllable structure, call duration or both, indicating a close connection of these AVU with the vocal motor system. Such connections might come from a recently found vocalization area in the ventrolateral pontine brainstem, lying close to the AVU in the present study (Fig. 14; Hage & Jürgens, 2006). It is proposed that this area serves as a vocal pattern generator for frequency-modulated call types such as trill vocalizations. The position of AVU adjacent to the vocalization area and the similar activity properties to self-produced vocalizations indicate that these

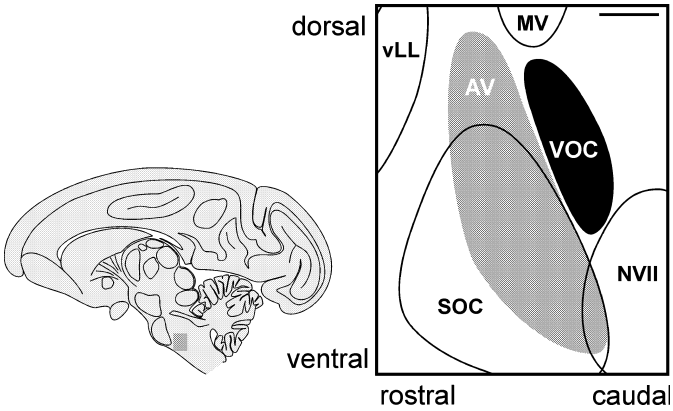

FIG. 14. Sagittal section through the squirrel monkeys' brain (lateral 1.8–3.8 mm), indicating the relative position of most of the recorded AVU (AV) and the recently found vocalization area (VOC; Hage & Jürgens, 2006). MV, motor trigeminal nucleus; NVII, facial nucleus; SOC, superior olivary complex; vLL, ventral nucleus of the lateral lemniscus. Scale bar, 500 μm.

AVU might be part of a vocal motor area with direct input from the auditory system.

Further support for a close connection between the AVU and the vocalization pathway at the level of the pontine brainstem comes from the fact that the ventrolateral pontine vocalization area is the highest structure in the descending vocal motor system reflecting in its neuronal activity trill-syllable structure (Hage & Jürgens, 2006). This property is not found in the next higher centre of the vocalization pathway, the periaqueductal grey (Düsterhöft *et al*., 2004). Furthermore, the vocalization area in the ventrolateral pontine brainstem has connections to all cranial motoneuron pools involved in phonation (Thoms & Jürgens, 1987; Li *et al*., 1993a,b; Hannig & Jürgens, 2006). This means that complex changes in vocal structure involving more than one motoneuron pool (e.g. changes in intensity, timing or duration) can be controlled by this area. This, however, is an essential requirement for the control of the above-mentioned audio–vocal reflexes (Suzuki & Sasaki, 1977; Sapir *et al*., 1983; Nonaka *et al*., 1997).

Broader implications

The findings of the present study indicate that the auditory system is modulated by the vocalization pathway and vice versa. Such reciprocal relationships seem to reflect a general principle of sensory–motor interactions as similar sensory–motor integrations are found in the visuomotor system. On the one hand, visual stimuli in the periphery of the visual field may initiate eye movements toward the signal. On the other hand, eye movements may influence visual input, for instance, in the form of an inhibition of visual input during and immediately before a saccade (Diamond *et al*., 2000). Similar to the audio–vocal integration, visuomotor integration can take place at different levels of the brain, with some of the processes already occurring at the lower brainstem level (Thilo *et al*., 2004) as we show here, for the first time, for audio–vocal interactions.

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Abbreviations

AVU, audio–vocal neurons; EAVU, excited audio–vocal neurons; FRP, pontine reticular formation; IAVU, inhibited audio–vocal neurons; LL, lateral lemniscus; LSO, lateral superior olive; MSO, medial superior olive; MTB, medial trapezoid body; OCS, olivocochlear system; PETH, peri-event time histogram; PLA, paralemniscal area; POR, periolivary region; PSTH, peri-stimulus time histogram; SOC, superior olivary complex; SPL, sound pressure level; vLL, ventral nucleus of the lateral lemniscus.

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