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Responses of primate frontal cortex neurons during natural vocal communication

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Miller CT, Thomas AW, Nummela SU, de la Mothe LA. Responses of primate frontal cortex neurons during natural vocal communication. *J Neurophysiol* 114: 1158–1171, 2015. First published June 18, 2015; doi:10.1152/jn.01003.2014.—The role of primate frontal cortex in vocal communication and its significance in language evolution have a controversial history. While evidence indicates that vocalization processing occurs in ventrolateral prefrontal cortex neurons, vocal-motor activity has been conjectured to be primarily subcortical and suggestive of a distinctly different neural architecture from humans. Direct evidence of neural activity during natural vocal communication is limited, as previous studies were performed in chair-restrained animals. Here we recorded the activity of single neurons across multiple regions of prefrontal and premotor cortex while freely moving marmosets engaged in a natural vocal behavior known as antiphonal calling. Our aim was to test whether neurons in marmoset frontal cortex exhibited responses during vocal-signal processing and/or vocal-motor production in the context of active, natural communication. We observed motor-related changes in single neuron activity during vocal production, but relatively weak sensory responses for vocalization processing during this natural behavior. Vocal-motor responses occurred both prior to and during call production and were typically coupled to the timing of each vocalization pulse. Despite the relatively weak sensory responses a population classifier was able to distinguish between neural activity that occurred during presentations of vocalization stimuli that elicited an antiphonal response and those that did not. These findings are suggestive of the role that nonhuman primate frontal cortex neurons play in natural communication and provide an important foundation for more explicit tests of the functional contributions of these neocortical areas during vocal behaviors.

primate frontal cortex; marmosets; antiphonal calling; vocal communication; natural behavior

HUMAN AND NONHUMAN PRIMATES (hereafter NHP) evolved intricate vocal communication systems to exchange information with conspecifics and efficiently navigate their respective social landscapes (Seyfarth and Cheney 2010). However, substantive differences are evident in the nature of these respective communication systems. Characteristic features of human language, such as semantic content and syntax, are rudimentary in NHPs (Marler 1985; Seyfarth and Cheney 2010). Likewise, some of the ontogenetic vocal learning mechanisms critical to human language acquisition appear limited or absent among our nonhuman cousins (Egnor and Hauser 2004). The source of these behavioral differences at the neural level is not well understood, but given the overall similarities in the basic neocortical architecture of all NHPs (Chaplin et al. 2013), key changes to aspects of the underlying neural circuitry likely occurred since diverging with our last common NHP ancestor (Schenker et al. 2005). One neocortical region that has undergone considerable change over primate evolution is frontal cortex (Semendeferi et al. 2001), a substrate known to be involved in many complex aspects of human cognition and communication (Fuster 2008; Gabrieli et al. 1998; Miller and Wallis 2012). Although aspects of communication signal processing in frontal cortex appear shared across humans and other NHPs (Averbeck and Romanski 2006; Cohen et al. 2007; Gifford et al. 2005; Plakke et al. 2013a; Romanski and Averbeck 2009; Romanski et al. 2005), the role of this neocortical substrate in NHP vocal production is more controversial. Much of the data on frontal cortex function during NHP vocal communication comes from studies of restrained animals (Averbeck and Romanski 2006; Cohen et al. 2007; Gifford et al. 2005; Romanski et al. 2005). Studies of NHP frontal cortex as individuals actively communicate with each other may lend important insight into the mechanisms underlying the observed differences in vocal behaviors across our Order.

Over the past three decades, researchers argued that, in contrast to humans, frontal cortex plays a relatively limited role in NHP vocal production (Deacon 1997; Fitch 2010; Jurgens 2002; Simonyan and Jurgens 2005). Two lines of evidence are frequently cited in support of this theory. First, neurons in both primate subcortical motor nuclei, such as periaqueductal gray (PAG), and cingulate cortex exhibit vocal-motor related changes in activity (Jurgens 2002; Larson and Kistler 1986). Complementary studies found that microstimulation of these neural substrates resulted in subjects emitting vocal utterances (Jurgens 2002; Jurgens and Ploog 1970). Second, lesions to lateral frontal cortex in monkeys did not result in permanent changes in vocal behavior; quantitative changes were only evident in the first few days or weeks (MacLean and Newman 1988). However, since similar effects with microstimulation in PAG and lesions to frontal cortex have been reported in humans (Jurgens 2002; Kertesz and McCabe 1977), the involvement of subcortical motor nuclei for vocal production does not preclude a role for frontal cortex. In fact, several recent experiments suggest that frontal cortex may contribute to some aspects of vocal production (Coude et al. 2011; Gemba

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et al. 1999; Hage and Nieder 2013; Miller et al. 2010a; Simoes et al. 2010). Microstimulation of ventrolateral prefrontal cortex area 44 in rhesus monkeys, for example, results in orofacial movements (Petrides et al. 2005). Resolving this long-standing debate requires more direct evidence of frontal cortex neuronal response properties during natural vocal production.

To address this issue we recorded the activity of single frontal cortex neurons in common marmosets (*Callithrix jacchus*) during naturally occurring long-distance vocal exchanges known as antiphonal calling (Fig. 1, *A* and *B*). This vocal behavior is ideally suited for neurophysiological experiments aimed at characterizing the activity of NHP frontal cortex neurons during natural, active communication for at least the following four reasons. First, this facet of marmoset communication has been extensively studied at the behavioral level (Chow et al. 2015; Miller et al. 2009a,b; Miller and Thomas 2012; Miller and Wang 2006; Morrill et al. 2013; Roy et al. 2011), providing a critical foundation for neurophysiological experiments. Second, these vocal exchanges are characterized by the reciprocal exchange of their species-typical phee calls (Fig. 1*B*) between visually occluded conspecifics (Miller and Wang 2006; Takahashi et al. 2013) (Fig. 1*A*). As such, marmosets serve as both a signal producer and signal receiver during these natural vocal exchanges, allowing the study of vocal signal perception, vocal-motor production, and any related sensory-motor interactions within individual neurons during the same behavior. During these vocal interactions, marmosets only produce phee calls (Miller and Wang 2006; Takahashi et al. 2013), making it possible to directly compare neural activity in response to the same call type as both a producer and receiver. Third, antiphonal calling is a sophisticated communication behavior. Marmosets exert control over the timing and occurrence of the behavior by monitoring both the occurrence of interfering noise and the behavior of other marmosets (Roy et al. 2011). Moreover, this capacity for social monitoring and vocal control is learned during ontogeny (Chow et al. 2015). Fourth, we developed a novel interactive playback paradigm that generates "virtual monkeys" that directly engage marmosets in these natural vocal exchanges (Miller et al. 2009a) that can be combined with techniques for recording single neuron activity in freely moving marmosets (Eliades and Wang 2008a).

Previous functional neuroanatomy studies of marmoset vocal behavior suggested that particular areas of frontal cortex are involved vocal-signal processing and vocal-motor production (Miller et al. 2010a; Simoes et al. 2010). This pair of experiments quantified immediate early gene (IEG) expression in freely moving animals as they engaged in antiphonal calling interactions, as well as the individual sensory and motor components that comprise the behavior. Data indicated that ventral prefrontal (areas 47, 45, 8av) and premotor (area 6v) cortex exhibited increased IEG expression during vocal-signal processing (Miller et al. 2010a), while IEG expression increased in dorsal premotor cortex (area 6d) during vocal-motor production (Miller et al. 2010a; Simoes et al. 2010). Building on these findings, we placed microelectrode recording arrays across these areas of marmoset frontal cortex (Fig. 1, *C* and *D*) and recorded the activity of single neurons throughout these populations. The primary aim of the current experiment was to test whether single neurons in these areas of frontal cortex exhibit changes in neural activity during either vocal-signal processing and/or vocal-motor production in a largely uncontrolled test environment characteristic of natural communication.

MATERIALS AND METHODS

Subjects. Three adult common marmosets (*Callithrix jacchus*) served as subjects in these experiments. Both B01 and R01 were male, while F01 was female. We recorded neural activity from two microelectrode arrays in subject B01. The array in the left hemisphere of B01 was centered in area 6v, while the second array was centered in area 6d in the right hemisphere. Subject R01 contributed data from a single array placed in the right hemisphere centered in areas 45 and 8av. Subject F01 had a single array placed in the left hemisphere centered in area 6d and the most rostral electrodes in 8ad. This positioning was similar to the dorsal array placed in subject B01. These locations were chosen based on previous work showing an increase in the expression of the IEG cFos during vocal-signal processing and/or vocal-motor production during antiphonal calling (Miller et al. 2010a). All animals were group housed, and experiments were performed in the Cortical Systems and Behavior Laboratory at University of California San Diego (UCSD). Experiments were approved by the UCSD Institutional Animal Care and Use Committee.

Behavioral paradigm. All recordings took place in a 4 × 3 m Radio-Frequency Shielded testing room (ETS-Lindgren). The testing room was organized with two rectangular tables positioned at opposite ends of the room 5 m apart. A cloth occluder positioned was equidistant between the two tables. A speaker (Polk Audio TSi100, frequency range 40-22,000 Hz) was positioned on one table, while the subject was placed on the other table. All vocal signal stimuli were broadcast at 80–90 dbSPL measured 1 m in front of the speaker. A directional microphone (Sennheiser, model ME-66) was placed 0.5 m in front of the subject to record all vocalizations produced during a test session. During experiments subjects were placed in a test cage (32 × 18 × 46 cm) constructed of plastic mesh on the front and back that allowed the animals to climb and jump freely along these walls. Because of the natural movements of subjects, the relative loudness of the stimulus and spatial position from which stimuli were broadcast did vary throughout these experiments.

All data were recorded during natural vocal communication behaviors. We employed interactive playback software to engage subjects in their naturally occurring antiphonal calling behavior (Miller et al. 2009a; Miller and Thomas 2012). Similarly to other NHP contact calls, the marmoset phee call produced during antiphonal calling exchanges communicates the spatial position and a myriad of social information between visually occluded conspecifics (Miller et al. 2010b; Miller and Thomas 2012; Miller and Wang 2006). This software was designed to both effectively mimic antiphonal calling in marmosets and allow some degree of experimental control over aspects of the vocal behavior. Rather than present vocalization stimuli at predetermined intervals similarly to traditional playback experiments, the timing of stimulus presentation in this paradigm was based on subjects' behavior. A more detailed description of the interactive playback paradigm for marmosets can be found elsewhere (Miller et al. 2009a; Miller and Thomas 2012); here we briefly describe the overall procedure employed during these experiments.

In each recording session, stimuli were phee calls produced by a single marmoset previously recorded during naturally occurring antiphonal calling interactions. Based on earlier work showing acoustic differences in phee calls based on the context in which they are produced (Miller et al. 2010b), we distinguished between phee calls produced in response to a conspecific's phee ("Antiphonal") and those produced spontaneously ("Independent"). The interactive playback software was designed to broadcast these stimulus classes, Antiphonal and Independent, at different intervals relative to subjects' behavior. Each time a subject produced a phee call, an

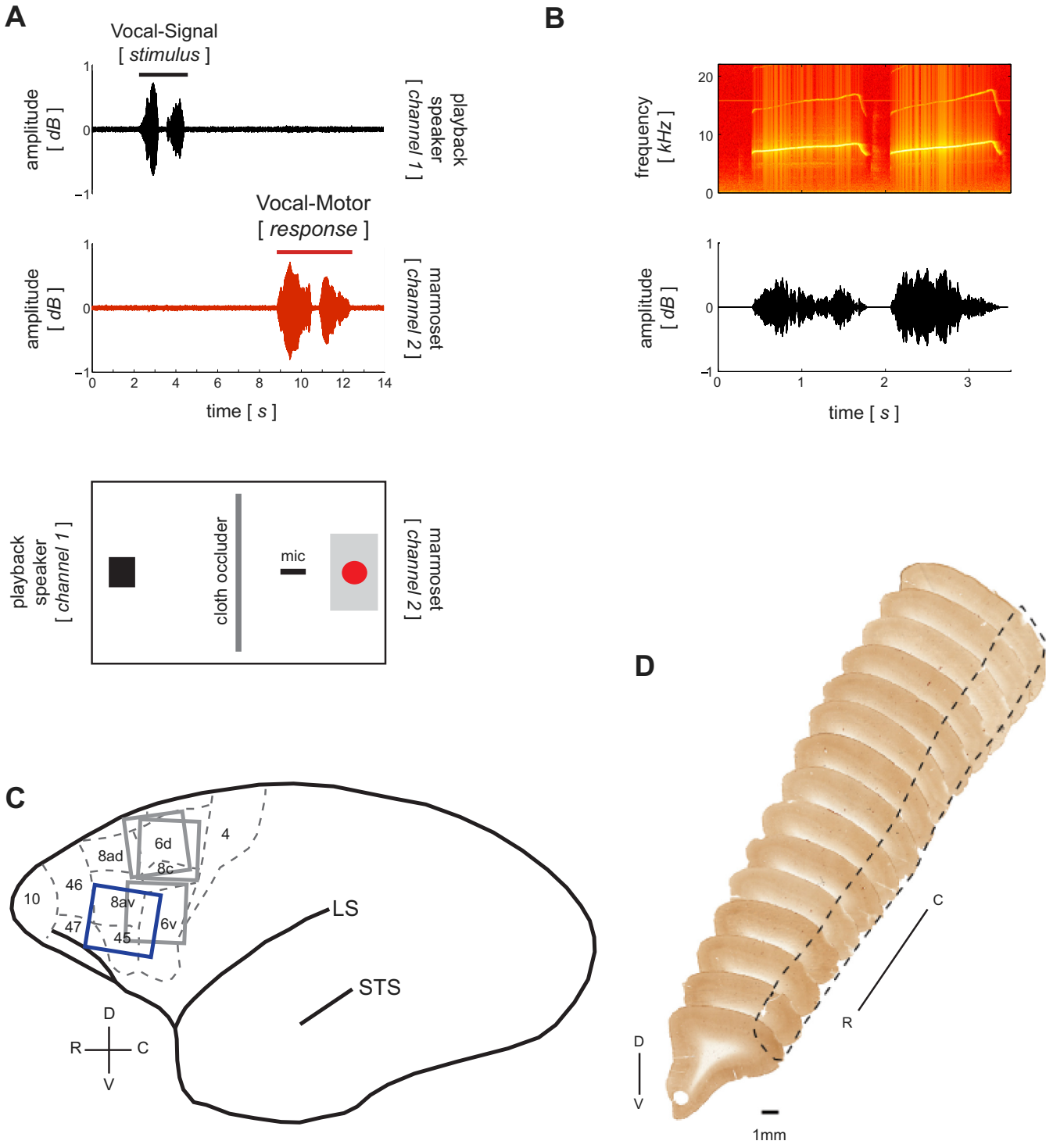


Fig. 1. *A*: exemplar recording of an antiphonal calling exchange between two marmosets. The phee call shown on *channel 1* (*top*) is the stimulus broadcast by the interactive playback software employed in this study. The phee call on *channel 2* is the antiphonal call response produced by the subject. The schematic of the experimental setup is shown below. Subjects (red circle) were positioned on the opposite side of a test chamber from a speaker (black square). A cloth occluder was positioned in the middle of the room equidistant between the subject and speaker. A microphone (mic) was positioned in front of the subject. *B*: spectrogram (*top*) and amplitude waveform (*bottom*) of a representative 2-pulse phee call. *C*: schematic drawing of marmoset cortex and the position of the 4 microelectrode recording arrays in frontal cortex. The blue square indicates the location of the sections plotted in *D*. Cytoarchitectural boundaries in marmoset frontal cortex are shown (Paxinos et al. 2012). LS, lateral sulcus; STS, superior temporal sulcus. *D*: serial frontal cortex sections for monkey R01. A dashed line shows the location of the electrode array from rostral-caudal. A vertical line provides an orientation for dorsal (D) and ventral (V) orientations, while the rostral (R)-caudal (C) axis is shown along the edge of the stack of individual sections.

Antiphonal phee call stimulus was broadcast 2–4 s following call offset. Bouts of antiphonal calling occurred when subjects alternated an antiphonal call response with a stimulus presentation consecutively. Independent phee call stimuli were broadcast if subjects produced no phee calls for 45–60 s. The aim of broadcasting Independent stimuli was to induce calling in subjects.

Phee calls produced by subjects in these experiments were categorized as either Antiphonal or Independent. An Antiphonal call would be any instance the subject produced a phee within 10 s of hearing a stimulus. An Independent call was classified as any phee produced >10 s after a stimulus presentation. These designations were based on previous studies reporting that phee calls produced within 10 s of a conspecific call were typically produced in response to the preceding call, while calls produced after this time period were generally vocalizations produced to elicit a response (Miller and Wang 2006). Experimental tests showed that only phee calls produced within 10 s of subjects calls were perceived as a response by marmosets and elicited high levels of antiphonal calls. Phee calls produced after this time were significantly less likely to result in a response, suggesting that this behavioral cue is perceptually salient and likely communicates a conspecific's intent to directly engage in a reciprocal vocal exchange (Miller et al. 2009a).

Neurophysiological recording procedures. Prior to the placement of the electrode arrays and initiation of the neurophysiology experiments, all subjects underwent surgery to implant an acrylic head cap and stainless steel head posts. During this surgery, the lateral sulcus as well as the rostral and lateral edges of frontal cortex were visible through the skull and marked. We were able to later use the markings on the skull made during surgery to triangulate the desired location of frontal cortex when placing the microelectrode array. The head posts, screws, electrode microarrays, and dental acrylic were not MRI compatible, thus precluding the use of structural MRI prior to and during neurophysiological recordings to determine the location of the microelectrode arrays in frontal cortex. However, the subsequent histological analysis performed on each subject's brain provided precise information about which regions of frontal cortex were recorded in these experiments.

We recorded neural activity with a Warp16 electrode array (Neuralynx, Bozeman, MT). The Warp16 comprises 16 independent guide tubes that house tungsten electrodes in a 4 × 4 mm grid. Since the arrays are positioned on the surface of the brain, electrodes are lowered perpendicularly to the laminar surface of neocortex. Individual electrodes in the Warp16 were advanced incrementally over the course of the experiment by restraining animals in a monkey chair. A calibrated Warp Drive pusher was attached to the end of each guide tube, and each respective electrode was advanced 10–20 μm twice a week. The Warp16 array was coupled with a tether to allow for freely moving behavior during recordings. Neural activity was digitized and sorted off-line. Based on previous studies using similar recording methods (Eliades and Wang 2008b), single units were determined based on the criteria that the unit have a signal-to-noise ratio ≥13 dB and <1% of interspike intervals <1 ms refractory period. Recording sessions typically lasted ~60–80 min. We recorded the activity of 366 neurons in marmoset frontal cortex during these experiments, of which 261 units were classified as isolated single neurons.

Perfusion, tissue processing, and reconstructions. The animal was anesthetized with ketamine, euthanized with pentobarbital sodium, and perfused transcardially with phosphate-buffered heparin solution followed by 4% paraformaldehyde. The brain was impregnated with 30% phosphate-buffered sucrose and blocked. The frontal cortex was cut at 40 μm in the coronal plane. Alternating sections were processed for cytochrome oxidase (Wong-Riley 1979), Nissl substance with thionin, vesicular glutamate transporter 2 (vGluT2), and neuronal tracers. Areas were determined by previously identified criteria (Paxinos et al. 2012). Figure 2 illustrates an example of tissue reconstructed with Nissl, CO, and vGLuT2. Lesions, electrode penetrations, and tracer injection sites were used to reconstruct the location of the electrode array with respect to the anatomical borders and confirm the location of electrode penetrations (Fig. 2). Images of the tissue were acquired using a Nikon eclipse 80i. These methods are similar to those employed in previous anatomical studies of marmoset neocortex (de la Mothe et al. 2006; 2012).

Data analysis. Analyses focused on the 188 of 261 isolated single neurons recorded during sessions in which subjects reached the following two behavioral criteria. First, all analyses were restricted to two-pulse phee calls. While marmosets naturally produce phee calls ranging from one to five pulses, the most commonly produced phee call comprises two pulses (Miller et al. 2010b). This allowed us to reduce the amount of acoustic variance in signal structure during sensory processing or motor production. Furthermore, marmosets establish a motor plan prior to vocal production for the number of pulses in the phee call (Miller et al. 2009b) that could affect aspects of vocal-motor related neural activity. Second, antiphonal calling comprises a sequence of a vocal-signal stimulus followed by a vocal-motor response (Fig. 1*A*). Each of these behavioral components also occur independently of this behavioral sequence. To facilitate analyses testing whether neural activity during the sensory and motor portions of the behavior were affected by behavioral context (i.e., Antiphonal vs Independent), we restricted analyses to only those sessions in which subjects engaged in at least 15 antiphonal calling

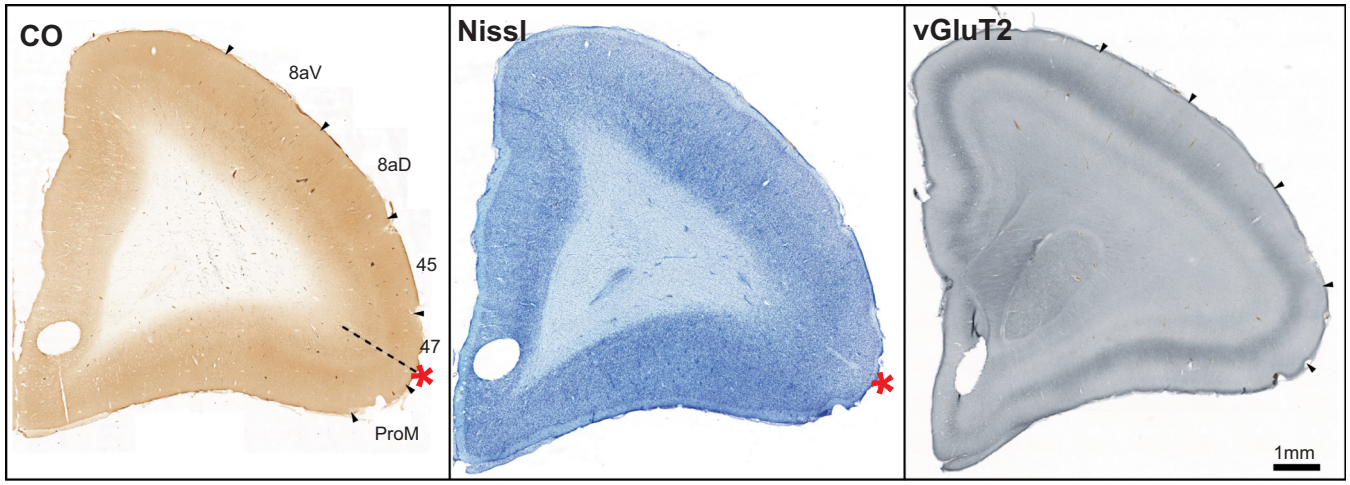
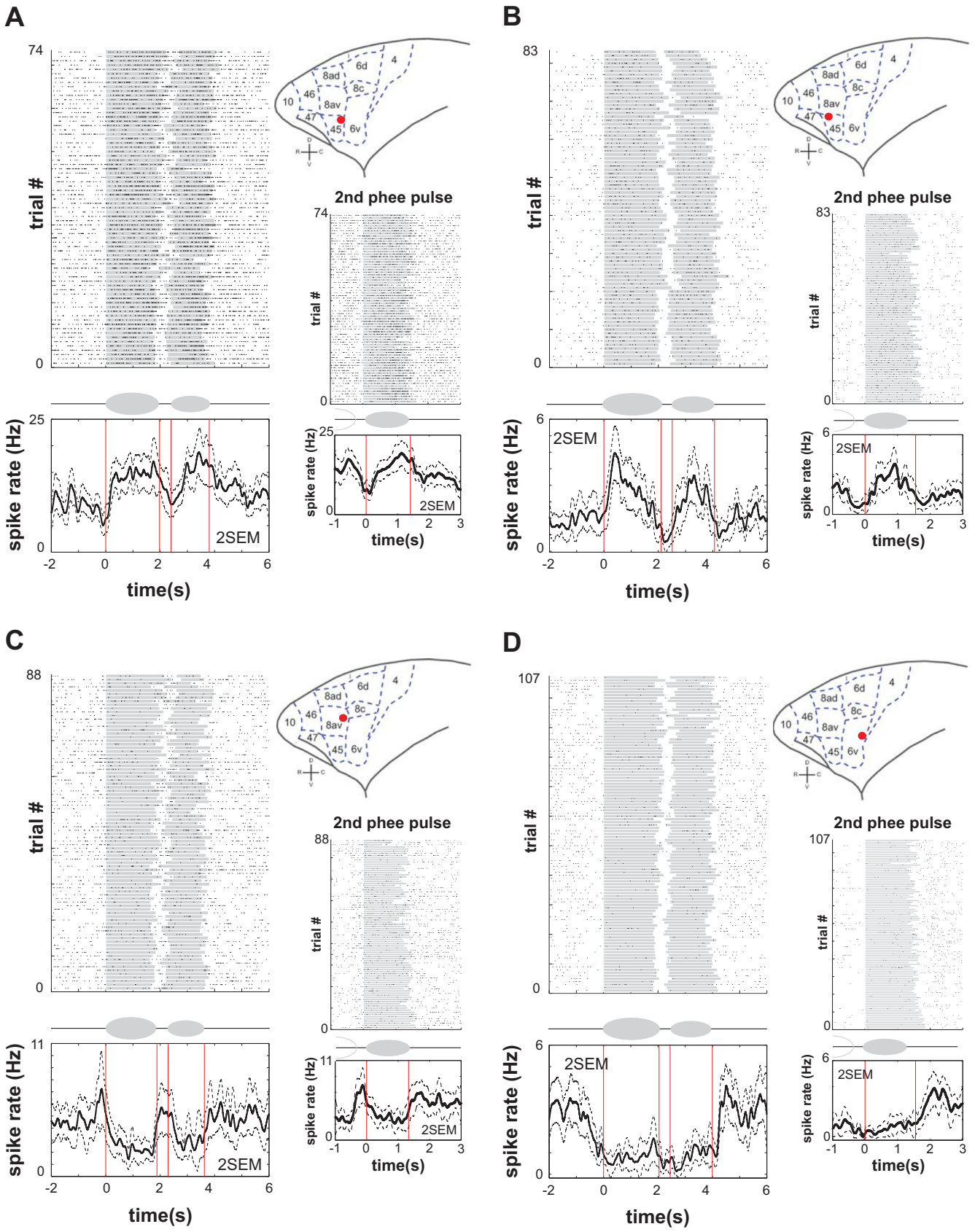


Fig. 2. Representative tissue for 3 stains (CO, Nissl, vGluT2) used in histological reconstruction of recording sites. The CO section shows the different areas of prefrontal cortex on the lateral surface. A dashed line and/or red star indicates an electrode tract. Boundaries of prefrontal cortex areas are shown: 8aV, 8aD, 45, 47, ProM.


A
B
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8c
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8av
47
45
6v

interactions, produced at least 15 independently produced phee calls, and were broadcast at least 15 phee stimuli that elicited no vocal responses (i.e., Independent). Subject B01 contributed 120 neurons to these analyses ($n = 96$ from the ventral array and $n = 24$ from the dorsal array). Subject R01 contributed 49 single neurons, all in ventral prefrontal and premotor cortex. And subject F01 contributed 19 single units in dorsal premotor cortex. The low number of neurons in this latter subject was because the animal consistently failed to reach the above behavioral criteria.

To test whether marmoset frontal cortex neurons were responsive during natural vocal communication, the following analyses were performed. We first analyzed whether the spike rate during the vocal-signal processing and vocal-motor production differed from baseline periods. We calculated separate baseline periods for the vocal-signal and vocal-motor periods on each trial, measured as the spike rate of the 1,000 ms prior to the onset of each behavioral component. For vocal signals, this baseline period occurred immediately before the onset of the stimulus. A Wilcoxon signed-rank test was used to compare the spike rate between the vocal-signal baseline and vocal-signal stimulus for each unit (alpha-level, $P < 0.05$). For the vocal-motor production, the baseline period was measured as 1,500-500 ms prior to vocal onset. This time window was used because we observed significant changes in neural activity in the 500 ms prior to vocal onset. We performed the following two analyses to test for vocal motor-related changes in neural activity. First, since changes in premotor neural activity were typically characterized by a rapid increase or decrease in spike rate just prior to vocal onset, we measured the interspike interval during the baseline premotor period (500-0 ms prior to vocal onset) and compared these distributions using a Kolmogorov-Smirnov (KS) test for each unit. This analysis was found to be most effective at capturing the relatively short changes in activity that occurred prior to vocal onset. Second, we compared the spike rate between the vocal-motor baseline and during vocal-motor production for each unit. Since the same neural activity from the vocal-motor baseline period was used in both analyses, the alpha-level was corrected to $P < 0.025$. A neuron was deemed vocal-motor responsive if either the significant change in activity occurred in either the premotor or motor periods of vocal production.

Neurons that exhibited a significant change in neural activity during the vocal-signal and/or vocal-motor periods of antiphonal calling were then analyzed to test whether these units showed a similar pattern of neural activity to when the respective behavioral components occurred independently. We compared the spike rate for the respective behavior component when it occurred during the Antiphonal and Independent contexts for each neuron (rank sum, alpha-level $P < 0.05$).

To move a step beyond describing the types of neural activity as sensory or motor related, we also measured how well the unit responses could classify phee calls using logistic regression. Three types of classification were attempted: *1*) distinguishing between vocal-signal processing and vocal-motor production of phee calls, *2*) distinguishing between vocal-signal processing in the Antiphonal and Independent contexts, and *3*) distinguishing between vocal-motor production in the Antiphonal and Independent contexts. Classification was only attempted for units that were isolated for at least 20 phee calls in each category. This number of calls allowed us to match the minimum 15 calls needed for previous analyses to train the classifier, while reserving at least five in which to test the fit of the model. Firing rates from 0 to 4 s from phee call onsets were fit using the Matlab function "glmfit" to a sigmoid defined by:

$$Y = \frac{1}{1 + e^{\wedge}\left[-\left(\beta_0 + \beta_1 * X\right)\right]}$$

Where Y indicates each category with the values 0 or 1, X is the neuron's firing rate in response to the phee call, and β_0 and β_1 are parameters. We used 75% of the responses to each category to construct firing rate distributions to train the classifier, and the remaining 25% of the responses were used to construct firing rate distributions for a test data set. To ensure the classifier had equal numbers of both categories, so that accuracy did not depend on the base rate of category occurrence, the training and test data sets were constructed by taking 5,000 draws from the firing rate distributions for each phee call category. To score the classifier, an estimate of the test dataset categories, $\hat{Y}_{test}$ was obtained by applying the sigmoid fit to the training data set to the test data set X_{test} and converted to categories by taking $\hat{Y}_{test} > 0.5$ to be 1 and $\hat{Y}_{test} \leq 0.5$ to be 0. Accuracy of the classifier was given by the sum of categories that matched the test data set, Y_{test}, divided by size of the data set. This procedure was repeated 500 times for each neuron to bootstrap median and 95% confidence intervals (CIs) of classifier accuracy.

A similar method was used to train a logistic regression classifier using the entire population of neurons, except that X is a $m \times n$ matrix, where m is the number of neurons, n is the number of firing rates in the test data set, and β_1 is a row vector with m entries. Importantly, the magnitude of each value in β_1 indicates how much influence each neuron has on the final categorization, which we refer to in the main text and legend of Fig. 5 as the "weight" of each neuron in this population classifier. Training and test data sets were constructed in the same manner as the single unit classifier, except each column of X is one draw from the firing rate distribution of each neuron. Neuron responses were also z-scored to ensure firing rate magnitudes across neurons were of similar scale. This procedure was performed 500 times to bootstrap the median and 95% CIs of classifier accuracy and to construct the distributions of population classifier accuracies shown in Fig. 5. To plot single unit classifier accuracies and population classifier accuracies, distributions were constructed using 20 evenly spaced bins for accuracies ranging from 0 to 1. For single units, we plotted the fraction of single units with median classifier accuracy in each bin, and the P value is given by a nonparametric sign-test. For the population classifiers, the distribution of accuracies was constructed from bootstrap described above, and the P value was given by the fraction of accuracies that performed below or equal to chance accuracy of 0.5.

RESULTS

We found neurons in marmoset frontal cortex were responsive during natural communication. Notably, the responses of individual neurons to phee call stimuli were relatively weak.

Fig. 3. Representative exemplar neurons recorded in marmoset frontal cortex during vocal production. Four individual neurons are shown (*A–D*). A raster (*top*) and mean spike rate (*bottom*) is shown for each neuron. A schematic of a phee call is shown in the middle in gray. Spike timing is aligned with the onset of the 1st pulse of 2-pulse phee calls (0 s). The gray bars in the raster indicate the timing of each pulse of the phee call for each trial, while vertical black lines on each horizontal trial represent an action potential. The vertical red lines in the peristimulus time histogram (PSTH) plot indicate the mean timing of each pulse in the phee calls produced during the recording session. The mean spike rate is plotted in a solid black line, while ± 2 SE is plotted in the dashed black lines. 2 SE bounds were generated using a Jacknife procedure that takes different parts of the data, 90% in each draw with a different 10th of trials left out each time, and computes the mean and standard deviation (STD) over those different parts. It then estimates the mean as the mean of those means, and the standard error of the mean as the STD of the means times square root(N) where N is 10 in this case. An *insert* of the same unit is shown to the *right*, highlighting neural responses during the 2nd pulse of each phee call. Spike times are aligned with the 2nd phee call pulse (0 s). Gray bars indicate the timing of the 2nd pulse only; otherwise data are plotted identically to the raster/PSTH for the entire 2-pulse phee call. Above this insert is a schematic drawing marmoset frontal cortex with the location of the individual neuron marked with a red dot.

Units did not show a consistent change in neural activity 2 SE above the baseline period. In contrast, individual neurons in marmoset frontal cortex did exhibit significant vocal motor-related changes in activity. Figure 3 plots four representative neurons that exhibited vocal-motor changes in activity. Neurons responsive during vocal-motor production tended to exhibit excitation (Fig. 3, *A* and *B*) or suppression (Fig. 3, *C* and *D*) over the duration of the vocalization. Units presented in Fig. 2, *A–C*, also demonstrate another property observed in this population. Changes in neural activity in these neurons were closely coupled to the onset and offset of individual phee pulses. A mean 111.0% change in activity (± 4.4 SE) from baseline to the premotor period onset then followed by a mean 94.4% (± 8.9 SE) sustained change in activity during phee production. Notably, the change in spike rate, inhibition or excitation, during the premotor period was typically the opposite of neural responses during vocal production. Units excited in the premotor phase were suppressed during motor production and vice versa. Only two of the 48 neurons showing a statistically significant change in activity during the premotor period did not follow this pattern. An exemplar neuron that did not show this pattern is shown in Fig. 3*D*. As evidenced in these exemplar neurons, considerable intertrial variability was evident in neural activity prior to vocal onset. The excitatory peak prior to vocal onset in the unit shown in Fig. 3*C* does not exceed 2 SE from baseline, while no suppression is evident in the premotor period for the unit shown in Fig. 3*B*. For both the Fig. 3, *B* and *C*, neurons, the change in neural activity between the premotor and motor periods was more pronounced preceding the onset of the second than first phee pulse. This pattern was also true across the population, as the mean percent change in activity during the premotor period for the second pulse was 132.1% (± 6.1 SE) followed by a 105.1% (± 0.9 SE) mean percent change in spike rate from the premotor to motor periods. Due to variability across neurons, explicating the physiological factors affecting neural responses over the transition between premotor and motor periods, as well as differences between the first and second phee pulses, will require a larger sample of neurons.

Analyses revealed that 54% of the 188 single neurons that exhibited a significant response to the vocalization stimuli and/or during vocal-motor production during antiphonal calling ($n = 101$). Single neurons exhibiting significant changes in activity during vocal-signal stimulus presentations or vocal-motor production did not exhibit clear anatomical specificity (Fig. 4*A*). Because of the relatively low numbers of units exhibiting sensory responses to vocalization stimuli, it would likely be difficult to discern a clear pattern. But the lack of specificity to an area of marmoset frontal cortex was evident for neurons exhibiting vocal motor-related changes in activity.

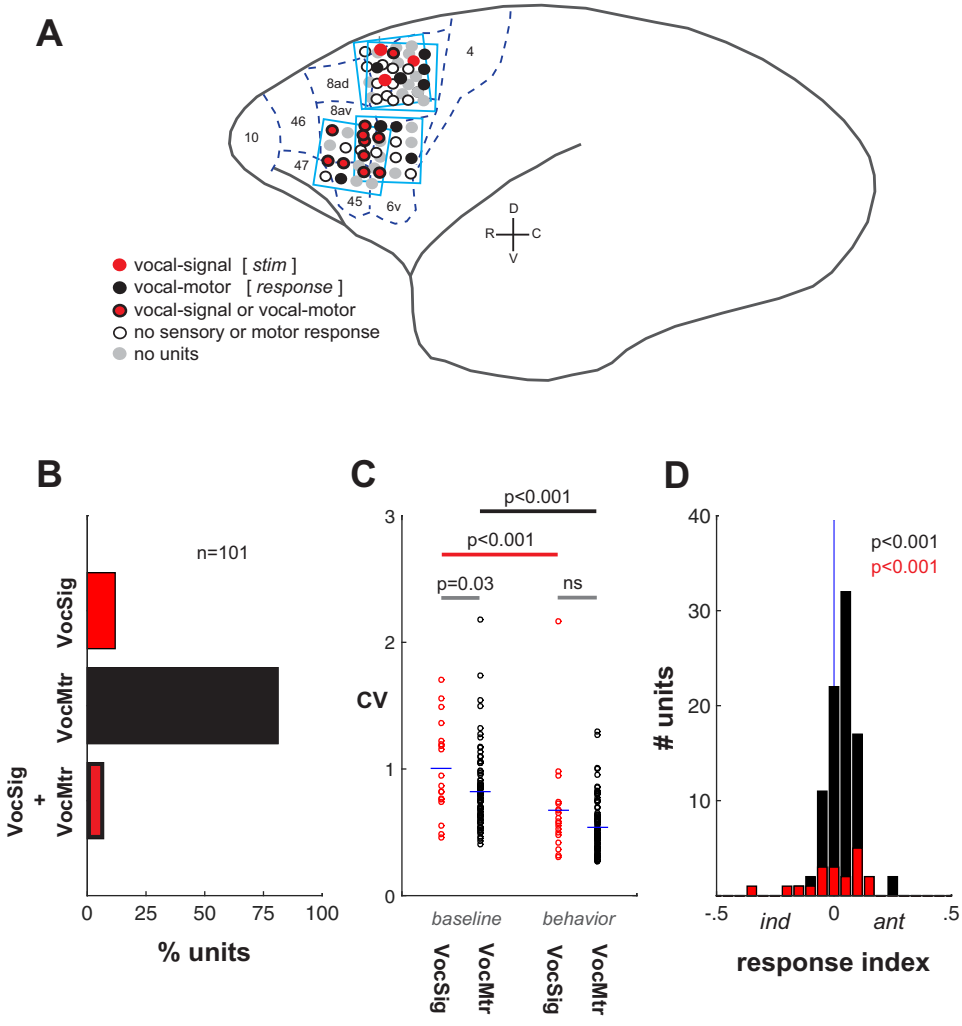

Fig. 4. *A*: a schematic drawing of marmoset cortex with the characteristic responses at each electrode location in frontal cortex. Red circle, vocal signal-responsive neurons only; black circle, vocal motor-responsive neurons only; red circle with black outline, vocal signal- and vocal motor-responsive neurons at this location; white circle, single units recorded but no vocal signal- or vocal motor-responsive neurons; gray circle, no units above neural and behavioral thresholds. The boundaries of the different frontal cortex regions are shown with dashed lines. The outline of each electrode array is shown in blue. *B*: bar graph plots the % of neurons exhibiting significant changes in neural activity to vocal-signal stimuli (vSig, black bar), during vocal-motor production (vMtr, red bar) or to both vocal-signals and vocal-motor (black bar with red outline). *C*: coefficient of variation (CV) of spike rate for each vocal-signal (vSig)- and vocal-motor (vMTR)-responsive neuron is shown during both the respective "baseline" and "behavior" periods. Blue lines indicate the mean CV. Statistically significant differences are indicated; n.s., not statistically significant. *D*: response-index comparing spike rate between the antiphonal and independent contexts. Red bars plot responses for vocal signal-responsive neurons, while black bars plot responses for vocal motor-responsive neurons. A positive (+) index indicates a stronger response when the behavior component occurred during antiphonal calling (ant), while a negative (−) index indicates a stronger response when the behavior component occurred independently (ind).

The majority of neurons recorded here exhibited significant changes in neural activity only during vocal-motor production of phee calls (Fig. 4*B*). While 81 of the responsive neurons were modulated only during the vocal-motor production, a mere 12 neurons exhibited a significant change in activity only during stimulus presentations of phee calls. An additional 8 units exhibited a significant change in neural activity for both vocal-signal and vocal-motor portions of the behavior. Given that previous studies of rhesus monkeys (Gifford et al. 2005; Plakke et al. 2013a; Romanski et al. 2005) and squirrel monkeys (Newman and Lindsley 1976) have all reported neurons responsive to vocalizations in the same areas of frontal cortex, the relatively weak sensory responses to vocal-signal stimuli across marmoset frontal cortex were somewhat surprising. Despite the weak responses to vocalization stimuli, we performed further analyses in parallel with vocal-motor responses to test whether effects at a population level might reveal key differences in neuronal patterns suggestive of why sensory responses were limited during this natural behavior.

One contributing factor for the relatively weak response to vocalization stimuli may be the variable state of frontal cortex. As these animals were freely moving, there were likely numerous endogenous and exogenous factors modulating the activity of frontal cortex neurons at the time stimuli were broadcast. However, because vocal-motor production is likely initiated by internal mechanisms, the timing of those events may not succumb to comparable fluctuations in ongoing neural activity. Previous studies of NHP cortical neurons reported that decreases in the variability of neural activity can be a meaningful correlate of important behavioral events (Churchland et al. 2011; Mitchell et al. 2009). To test whether similar effects occurred in this population of neurons, we calculated the coefficient of variation (CV) of the spike rate during the baseline period preceding vocal-signal stimulus broadcasts and vocal-motor production, as well as during each of these behavioral components. This analysis was performed by calculating the spike rate during the baseline and the behavioral event (vocal signal or vocal motor) on each trial for neurons exhibiting a statistical change in activity between these periods of antiphonal calling. We observed a decrease in variability during each of the behavioral components relative to the respective baseline (vocal signal: rank sum, $P < 0.001$; vocal motor: rank sum, $P < 0.001$; Fig. 4*C*). Analyses also indicate that the CV during the baseline periods was significantly higher prior to stimulus broadcasts than vocal production (rank sum, $P = 0.03$). This suggests that baseline variability differs prior to the onset of vocalization stimuli and vocal production and may potentially have an effect on neuron responses during those respective behavioral periods. The source of variance in baseline activity is difficult to ascertain in the current study, as it could arise from a number of exogenous and endogenous factors, including attention.

The vocal-signal and vocal-motor behavioral components examined here occurred during Antiphonal calling exchanges and Independent of this context. These behavioral differences afforded us to examine the effects of communication context on neural responses. Neurons exhibiting a significant response during one or both of the behavioral components in antiphonal calling showed no difference in activity when the respective component (vocal signal or vocal motor) occurred independently. In other words, a single neuron responsive to vocal-signal stimuli during antiphonal calling was similarly responsive to these stimuli when they did not elicit a vocal response. In fact, only one neuron showed a difference between these contexts for vocal signals and two neurons for vocal-motor activity. This suggests that the responses of individual neurons are not specific to the unique to contexts in which vocal exchanges occur, but to a broader range of natural communication contexts. To test whether differences in these contexts were evident at a population level, we calculated a response index comparing neural activity between each behavior component when it occurred independently and during antiphonal calling [(Antiphonal_SPKRate − Independent_SPKRate)/(Antiphonal_SPKRate + Independent_SPKRate)]. Analyses found that the population had a bias toward a stronger neural response during the antiphonal context than in the independent context (Fig. 4*D*). This was true for both vocal-signal processing (KS test, $P < 0.001$) and vocal-motor production (KS test, $P < 0.001$). These findings suggest that although individual neurons are likely to be responsive in both behavioral contexts, the antiphonal behavior is likely to elicit a somewhat stronger response across the population.

To further explore the role of these frontal neurons in coding aspects of vocal sensory processing and vocal motor production, as a population, we used logistic regression to test how well individual neurons, and the population of neurons could distinguish between three categories: *1*) vocal-signal processing and vocal-motor production of phee calls, *2*) vocal-signal processing in the Antiphonal and Independent contexts, and *3*) vocal-motor production in the Antiphonal and Independent contexts. As expected by the bias of neurons to exhibit changes in activity during vocal-motor production but not vocal-signal processing, several individual neurons could significantly distinguish between these two categories (Fig. 5*A*), and overall, the neuron population showed significantly greater accuracy than chance performance of 0.5 (sign-test, $P < 0.0001$). Although these units were not recorded simultaneously, a classifier using the population of neurons could be trained by drawing a test data set using Monte Carlo simulations of trials from each category (see MATERIALS AND METHODS). Classification using the population of neurons was highly significant ($P < 0.0001$) and nearly perfect (Fig. 5, *A*, *right*) and was a result of heavily weighting individual neurons with high classification performance. Although this outcome was expected due to the large number of units with greater activity during vocalizations, it validates the use of logistic regression to classify neural activity as belonging to different behavioral contexts. In contrast, by and large individual neurons were unable to distinguish between vocal-signal processing in the Antiphonal and Independent contexts (Fig. 5*B*, sign-test, $P = 0.15$); however, a classifier using the whole population of neurons was able to significantly distinguish between the two behavioral contexts ($P = 0.004$), with median performance comparable to median performance of the best single units, but with less variance. Individual units were not able to significantly distinguish calls produced in the Antiphonal and Independent contexts (Fig. 5*C*, sign-test, $P = 0.5$), and neither was a classifier using the entire population ($P = 0.11$). This indicates that despite the lower amount of neural activity observed in these frontal cortical units during vocal signal processing, as a whole the population of units distinguish

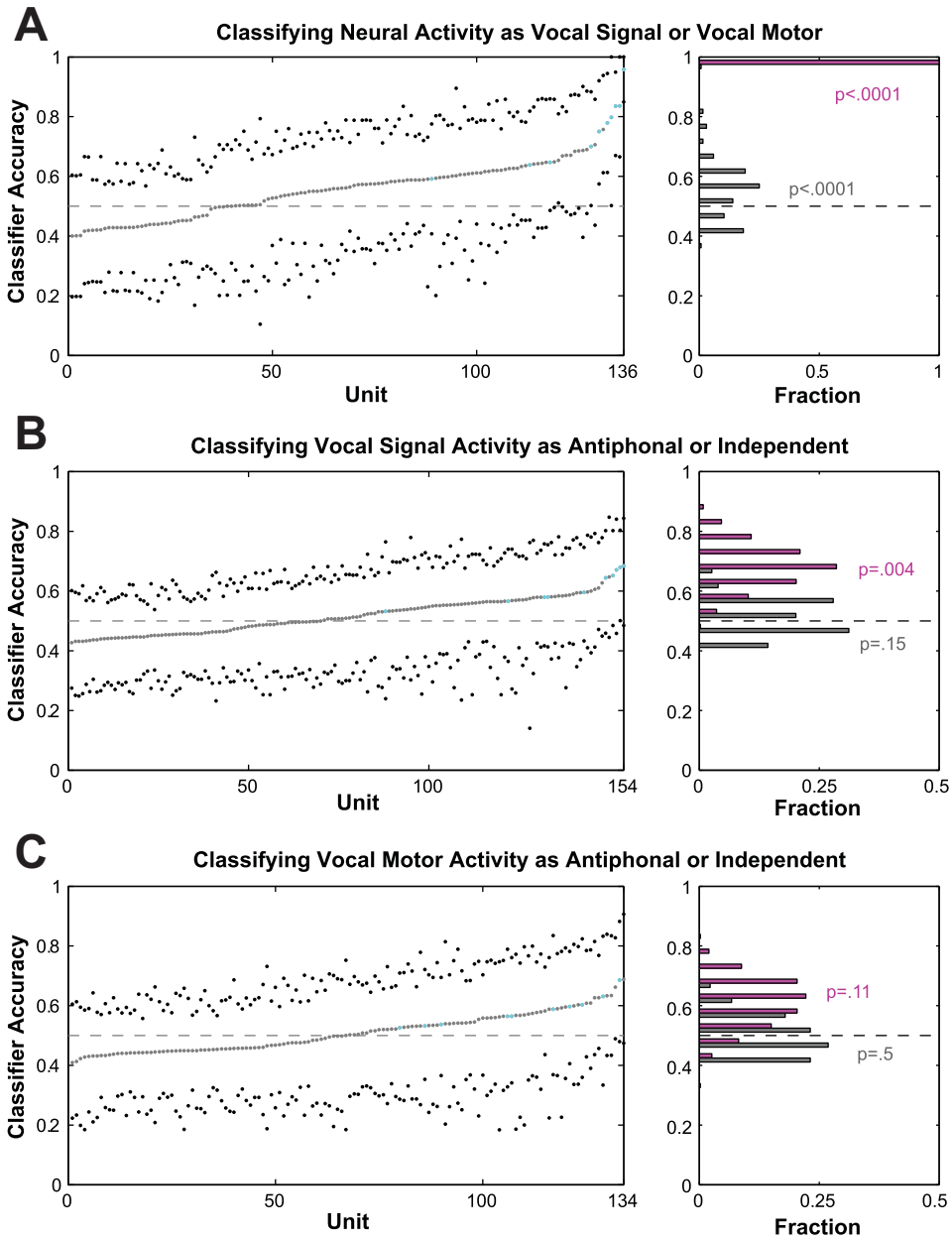

Fig. 5. Neural classification of phee calls. On the *left* side, median accuracy of logistic regression for single units to classify between vocal-signals and vocal-motor activity (*A*), vocal-signal processing between antiphonal and independent contexts (*B*), and vocal-motor production between antiphonal and independent contexts (*C*) using firing rates from 0 to 4 s from phee call onset for each category. Units are ordered from worst median performance to best. Error bars are 95% confidence intervals. The 10 single units weighted most by the median population classifier are highlighted in blue. On the *right* side, the distribution of median classification accuracy for the single units (gray) is compared with the distribution of population classifier accuracies (magenta) derived by a bootstrap procedure.

between the behavioral context with which the observed call will be used. However, it may be that only a small proportion of units in frontal cortex distinguish between the contexts, because in general, single units were unable to make these distinctions.

We observed neurons that were either excited or suppressed during vocal-motor production (Fig. 6*A*). Both of these neuronal classes were observed across areas of frontal cortex sampled here (Fig. 6*B*). It is notable that a population of vocal motor-responsive neurons occurred in ventral prefrontal cortex areas 8av and 45, the area corresponding to Broca's homolog. The exemplar neurons shown in Fig. 3, *A* and *B*, for example, were recorded in area 45, while the unit shown in Fig. 3*C* was recorded in area 8aV. We grouped spikes into 100 ms bins for each neuron in order to test whether differences in the timing of peak responses were evident across the population of neurons suppressed and excited during vocal production. Analyses revealed that suppressed vocal-motor neurons exhibited a shorter latency to peak response than excited vocal-motor neurons. Figure 6*C* plots the distribution of the latency to peak responses for both excitatory and suppressed vocal-motor neurons. The majority of suppressed vocal-motor neurons exhibited a peak response within the first 1 s following vocal onset (55%, mean = 1,295 ms). In contrast, 49% of excitatory vocal-motor neurons exhibited a peak response >2 s following vocal onset (mean = 1,910 ms). The differences in peak latency distribution between these neuronal types were statistically significant (rank sum, $P = 0.01$) and may reflect the functional role each plays in vocal production. Both classes of vocal-motor neurons exhibited the same broad pattern that spike rate variability significantly decreased during vocal production relative to baseline (suppressed: sign-rank, $P < 0.001$; excitatory: sign-rank, $P < 0.001$; Fig. 6*D*), suggesting that this characteristic of neural activity was independent of the type of neural response.

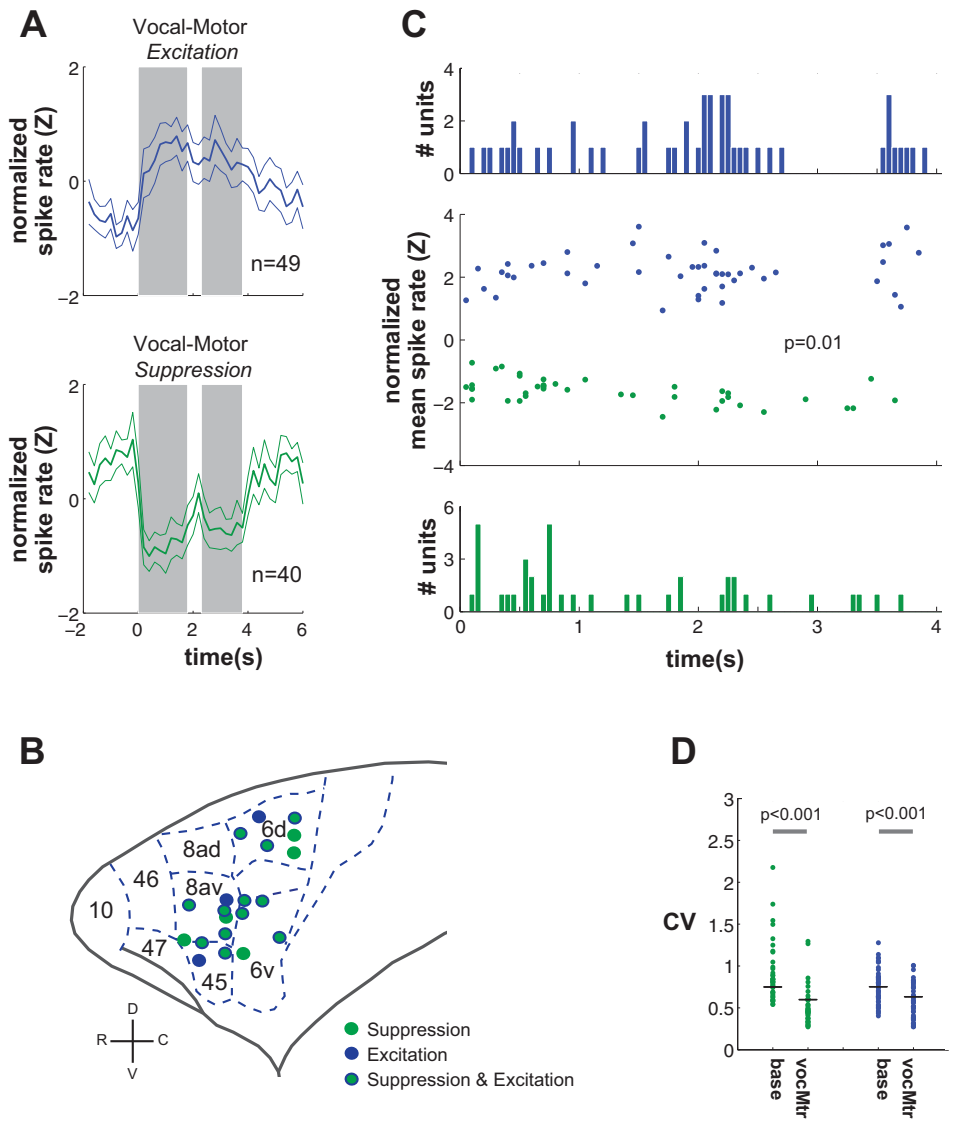

Fig. 6. Summary of results for vocal motor-responsive neurons. *A*: plots the mean (±2 SE) for the population of neurons exhibiting excitation (*top*, blue) or suppression (*bottom*, green) during vocal-motor production. *B*: a schematic drawing of marmoset frontal cortex with the locations of vocal motor-responsive neurons. Each recording site at which these neurons were found are shown and marked by the type of responses characterizing that location. Green, suppressed; blue, excited; green circle/blue border, neurons exhibiting either suppression or excitation were found. *C*: latency to peak response for both suppressed (green) and excited (blue) vocal-motor neurons is shown in a scatter plot in the middle. Spikes were grouped into 100 ms bins. Histograms plot the # of neurons at each latency for suppressed (*bottom*) and excited (*top*) vocal-motor neurons. *D*: plots the CV for suppressed (green) and excited (blue) vocal-motor neurons. The CV for the baseline (base) and during vocal production (vocMtr) is plotted for each class of neurons. Statistical significance is noted accordingly throughout the figure.

DISCUSSION

Communication is an inherently interactive process involving the exchange of signals between individuals (Dawkins and Krebs 1979; Guilford and Dawkins 1991; Miller and Bee 2012). Yet with a few notable exceptions (Eliades and Wang 2012; Eliades and Wang 2008b; 2003), neurophysiological experiments exploring the neural basis of vocal communication in NHP neocortex have been performed in chair restrained animals either passively listening to vocalizations or engaged in a conditioned behavioral task (Ghazanfar et al. 2005; Gifford et al. 2005; Newman and Wollberg 1973a,b; Perrodin et al. 2011; Plakke et al. 2013b; Romanski et al. 2005; Winter and Funkenstein 1973; Wollberg and Newman 1972). Building on studies reporting an increase in IEG expression in marmoset frontal cortex during natural communication (Miller et al. 2010a; Simoes et al. 2010), we recorded the activity of single neurons across the same neocortical areas in freely moving subjects engaged in this natural vocal behavior. Here we report that individual neurons in marmoset frontal cortex exhibited strong vocal motor-related changes in activity preceding and during vocal production, while vocal-signal stimuli elicited relatively weak responses. However, neural activity across the population of neurons during presentations of these vocalization stimuli could be correctly classified as either eliciting a subsequent antiphonal response or not. This suggests that while individual neurons were not driven by vocalization stimuli in this natural behavior, these populations do play some role in communication signal processing. Potential explanations for the observed differences between earlier studies of vocalization processing (Romanski and Averbeck 2009) and vocal-motor production (Coude et al. 2011; Hage and Nieder 2013) in restrained primates and data reported here during natural communication are discussed below. These data provide a foundation for future neurophysiological experiments and are suggestive that implementing more naturalistic behavioral paradigms are important for elucidating the neural basis of NHP vocal communication.

In contrast to previous experiments in primate frontal cortex (Romanski and Averbeck 2009), neurons in marmoset frontal cortex were not strongly driven by vocalization stimuli. The proportion of the population modulated by this aspect of vocal-signal processing in the current experiment (10%) was notably lower than in previous studies. Experiments in rhesus monkeys reported ~50–70% of vlPFC neurons as vocalization

responsive (Gifford et al. 2005), while ~20% of squirrel monkeys frontal cortex neurons exhibited significant responses to species-specific vocalizations (Newman and Lindsley 1976). There are at least three nonmutually exclusive explanations for these differences. First, we did not record from the equivalent areas of frontal cortex as in previous studies. Neurons in squirrel monkeys frontal cortex were sampled broadly throughout dorsal and ventral areas (Newman and Lindsley 1976), whereas the macaque studies indicate that vocalization responsive neurons are evident in an area of vlPFC (Gifford et al. 2005; Romanski et al. 2005). Each of these studies uses frontal sulci as landmarks for recording sites, which are lacking in the lissencephalic marmoset frontal cortex. However, histology from our study would indicate that we had considerable overlap with all of these regions, with the exception of the more anterior neurons recorded in dorsal squirrel monkeys frontal cortex. While more focused sampling in particular populations of marmoset cortex may be needed to reveal similar neuronal responses, neurons from the analogous neural areas were likely sampled in the current study. Second, it is possible that this pattern of results could result from species differences. Squirrel monkeys and marmosets are New World monkeys and separated from Old World monkeys, such as rhesus monkeys, by ~10–15 million years of evolution (Fleagle 1998). Despite this evolutionary divergence, much of the frontal cortex architecture has been conserved, though there is some indication that ventrolateral prefrontal cortex is relatively larger in rhesus monkeys than marmosets (Chaplin et al. 2013). Tracer experiments in marmoset frontal cortex suggest that, similarly to rhesus monkeys (Romanski et al. 1999a), marmoset prefrontal and auditory cortex share reciprocal anatomical connections (Reser et al. 2012). There is some indication that this pathway is less robust in marmosets than rhesus monkeys, but more work is needed. These data suggest that there may be some neuroanatomical differences between marmosets and rhesus monkeys that could affect responses to vocalizations but perhaps cannot solely account for the dearth of vocalization responses observed here.

The third possible explanation of the differences evident across these studies may relate to the behavioral context in which the respective experiments were performed. The implementation of a fixation or conditioned behavioral task in previous studies of vocalization responsive neurons in prefrontal cortex (Gifford et al. 2005; Plakke et al. 2013b; Romanski et al. 2005) likely served to control subjects' attention, and presumably underlying brain state. During natural communication, the timing of vocalizations is typically unpredictable. Therefore, a myriad of exogenous and endogenous factors could modulate neural activity at the time a call is heard and affect a single unit's response to the stimulus. The lower variation in baseline spike rate preceding vocal-motor production may occur because a motor plan is internally generated prior to the decision to initiate the action (Fig. 4*D*) (Churchland et al. 2011; Miller et al. 2009b; Zingale and Kowler 1987). An analogous process may not occur for vocal-signal processing because the timing of the stimuli lacks predictability. Behavioral context is known to affect the responses of frontal cortex neurons in a variety of conditioned tasks (Cromer et al. 2010; Duncan 2001; Rigotti et al. 2013; Stokes et al. 2013) and would presumably also be a significant factor in modulating neural responses during natural behavior. The classifier analysis presented here would support this conjecture. Neural activity during vocal-signal processing in marmoset frontal cortex could be correctly classified to a significant degree as either eliciting a subsequent Antiphonal response or not (Independent). Therefore, although individual neurons did not exhibit strong responses to vocalizations, their activity was modulated by the specific behavioral context. This was not a general feature of frontal cortex, however, since no difference was observed between these contexts for vocal-motor activity. The significance of dynamic population coding in primate frontal cortex may reflect the functional role that this area of neocortex plays in mitigating complex nuances that occur in complex primate behaviors (Mante et al. 2013), such as natural communication.

Marmoset frontal cortex neurons exhibited motor-related changes in activity during natural vocal production. These data would seem to contradict speculation that NHP frontal cortex plays a limited role in vocal production (Deacon 1997; Fitch 2010; Jurgens 2002). The specific functional contributions of these populations are difficult to ascertain from these data alone, but the timing of vocal-motor related responses may lend some insight. Units typically exhibited tight coupling between the timing of responses and the onset/offset of phee call pulses. Exemplar neurons shown in Fig. 3, *A–C*, demonstrate this trend. Each unit exhibits a change in neural activity just prior to the onset of each pulse, followed by a sharp change in response following pulse onset. Neurons suppressed during the premotor period were excited during motor production, while units exhibiting an excitatory response prior to pulse onset were inhibited while the pulse was produced. One interpretation of these data is that premotor activity is indicative of mechanisms for vocal control, while neural responses during vocal production reflect persistent feedback between frontal and auditory cortex (Eliades and Wang 2008b). For example, marmoset callers establish a motor plan prior to vocal onset for the acoustic structure of the phee call (Miller et al. 2009b) and avoid overlapping each other's vocalizations during antiphonal exchanges (Miller and Wang 2006; Takahashi et al. 2013). Furthermore, pairs of marmosets coordinate the timing of their antiphonal exchanges to avoid acoustic interference that might degrade signal efficacy (Roy et al. 2011). Alternatively, these responses could be related to orofacial movements that occur prior to and during vocal production, such as the mouth opening and closing. Microstimulation of ventrolateral prefrontal cortex neurons in rhesus monkeys evokes facial movements (Petrides et al. 2005). The observed population differences in the timing of peak response for suppressed and excited vocal-motor neurons may also be indicative of functional differences (Fig. 6*B*), but more experiments are needed. Extensive tests of marmoset frontal cortex with other motor tasks, as well as pharmacological, electrical, or optical manipulation of the substrate, are needed to more accurately gauge its functional contributions to NHP vocal production and relationship to subcortical motor nuclei.

The neurophysiological properties of vocal motor-responsive neurons in the current experiment contrast with recent studies of trained vocal production in rhesus monkeys (Coude et al. 2011; Hage and Nieder 2013). Similarly to these rhesus monkey experiments, we found that neurons in ventral prefrontal area 44/45, putative Broca's homolog, and ventral premotor cortex exhibit vocal motor-related changes in activity. How-

ever, our findings differ from these studies in two important respects. The rhesus monkey studies reported a significant change in neural activity only prior to vocal onset (~500–1,000 ms), whereas neurons recorded during natural communication in the current experiment exhibited pronounced changes in activity during vocal production (Figs. 3 and 6*A*). Furthermore, the vocal-motor activity in these previous studies occurred only during trials in which subjects produced a vocalization in response to the trained stimulus; vocalizations produced spontaneously (i.e., naturally) elicited no changes in neural activity. In the current study, we observed that neurons during antiphonal calling exhibited a similar pattern of activity during spontaneous calling, though at the population level neurons exhibited stronger changes in activity during the former vocal behavior (Fig. 4*B*). The discrepancy between these studies likely reflects differences in task demands and suggests that the neural activity observed in the rhesus monkey experiments may be related to the training paradigm rather than natural vocal production. Trained behaviors may not necessarily parallel key features of natural behaviors, particularly with respect to communication.

One key advantage of the paradigm employed here is that it allows the study of active communication in an NHP, but this model system is not without its shortcomings. When spatially separated and visually occluded, as in the current study, marmosets typically only produce a single call type, the phee call (Miller and Wang 2006; Takahashi et al. 2013). As the marmoset vocal repertoire comprises numerous other calls (Bezerra and Souto 2008), a more comprehensive study of multiple vocalizations would be necessary to fully characterize the neural basis of vocal communication in marmoset frontal cortex. Similar studies of multiple vocalization types have been performed in marmoset auditory cortex (DiMattina and Wang 2006; Eliades and Wang 2013; Kajikawa et al. 2008; Wang et al. 1995). Likewise, antiphonal calling is an active communication behavior, but it is possible that idiosyncrasies of these exchanges are not representative of all primate vocal interactions. Comparing neural activity in a more diverse set of vocal behaviors would be necessary to fully capture the role of primate frontal cortex in active communication exchanges. And finally, the uncontrolled movements of the marmosets in these experiments almost certainly introduced a myriad of variability into subjects' behavior and neural activity. Although this variance is likely typical of a naturally behaving NHPs, it also complicates identifying clear neural responses during communication behaviors. Studies comparing neural activity in both restrained and freely moving animals are needed to address this issue. These broader issues with this model system limit interpretations of the data presented here.

Primate neocortex comprises distinct dorsal and ventral processing pathways (Goodale and Milner 1992; Ungerleider and Mishkin 1982). Although this dual-stream model was originally described for the visual system, evidence of a similar pathways was first reported in the NHP auditory system (Rauschecker and Tian 2000; Recanzone and Cohen 2010; Romanski and Goldman-Rakic 2001; Romanski et al. 1999b; Tian et al. 2001), followed by more recent evidence for speech and language in human brains (Hickok and Poeppel 2007; Poeppel and Hickok 2004). Mechanisms in the ventral stream are thought to underlie increasingly complex aspects of vocal signal processing and recognition (Gifford et al. 2005; Romanski et al. 2005), while the dorsal stream functions to integrate sensory input to a vocal-motor output in NHP vocal communication systems (Rauschecker 2012; Rauschecker and Scott 2009). Anatomical evidence shows that these streams originate in auditory cortex and terminate in frontal cortex of NHPs (Rauschecker and Scott 2009; Romanski et al. 1999b). With some exceptions (Cohen et al. 2009), relatively few data from frontal cortex have identified functional differences between dorsal and ventral populations at this stage of the auditory pathway. This is particularly true for studies of vocal communication, where data are only available from the ventral pathway (Romanski and Averbeck 2009). Although the current experiment recorded neurons in ventral and dorsal frontal cortex regions during communication exchanges, we did not observe differences in neural responses between these anatomical locations. As such, our data are relatively agnostic with respect to dual-stream models. It is possible that other aspects of communication exchanges, auditory feedback, and/or vocal articulation could elicit more robust neural responses and provide evidence of functional specialization (Rauschecker 2012). The absence of clear anatomical differences in the current study may reflect issues with the nature of these experiments, as discussed above, rather than a substantive challenge to dual-stream models.

We recorded neurophysiological responses of marmoset frontal cortex neurons during natural vocal communication. Units recorded in this context exhibited several differences from analogous studies performed in restrained monkeys either passively listening or performing a behavioral task. These distinctions may result, at least in part, from differences in the behavioral contexts in which neurons were recorded (Cromer et al. 2010; Duncan 2001; Rigotti et al. 2013; Stokes et al. 2013). Given that communication is defined by the exchange of information between conspecifics (Dawkins and Krebs 1979; Guilford and Dawkins 1991; Miller and Bee 2012), more traditional neuroscientific approaches may not reflect the interactive nature of natural communication, and potentially other social behaviors. As these data are relatively preliminary, further experiments are needed to more explicitly test the extent to which these contextual differences, or other factors, may underlie the pattern of neural responses reported here and explicate the functional role that these populations play in vocal communication. While the current study employed a relatively simple interactive playback paradigm to engage subjects in communication exchanges, more sophisticated versions of this paradigm that manipulate aspects of the vocal signal and/or vocal behavior (Miller et al. 2009a; Miller and Thomas 2012) can also be implemented in neurophysiological experiments to test their effects on frontal cortex in future studies. Given that neurophysiological studies of vocalization processing are possible in both restrained (DiMattina and Wang 2006) and freely moving (Eliades and Wang 2008a) marmosets, this species represents an ideal system in which to further examine the neural mechanisms underlying NHP vocal communication.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: C.T.M. conception and design of research; C.T.M., A.W.T., and L.A.d.l.M. performed experiments; C.T.M., A.W.T., S.U.N., and L.A.d.l.M. analyzed data; C.T.M., A.W.T., and L.A.d.l.M. interpreted results of experiments; C.T.M., S.U.N., and L.A.d.l.M. prepared figures; C.T.M. drafted manuscript; C.T.M., A.W.T., S.U.N., and L.A.d.l.M. edited and revised manuscript; C.T.M., A.W.T., S.U.N., and L.A.d.l.M. approved final version of manuscript.

REFERENCES

Averbeck BB, Romanski LM. Probabalistic encoding of vocalizations in macaque ventral lateral prefrontal cortex. *J Neurosci* 26: 11023–11033, 2006.

Bezerra BM, Souto A. Structure and usage of the vocal repertoire of Callithrix jacchus. *Int J Primatol* 29: 671–701, 2008.

Chaplin T, Yu H, Soares J, Gattass R, Rosa MGP. A conserved pattern of differential expansion of cortical areas in simian primates. *J Neurosci* 18: 15120–15125, 2013.

Chow C, Mitchell J, Miller CT. Vocal turn-taking in a nonhuman primate is learned during ontogeny. *Proc Roy Soc B* 282: 210150069, 2015.

Churchland AK, Kiani R, Chaudhuri R, Wang XJ, Pouget A, Shadlen M. Variance as a signature of neural computations during decision making. *Neuron* 69: 818–831, 2011.

Cohen YE, Russ BE, Davis SJ, Baker AE, Ackelson AL, Nitecki R. A functional role for the ventrolateral prefrontal cortex in non-spatial auditory cognition. *Proc Natl Acad Sci USA* 106: 10045–10050, 2009.

Cohen YE, Theunissen FE, Russ BE, Gill P. Acoustic features of rhesus vocalizations and their representation in the ventrolateral prefrontal cortex. *J Neurophysiol* 97: 1470–1484, 2007.

Coude G, Ferrari PF, Roda F, Marensi M, Borelli E, Veroni V, Monti F, Rozzi S, Fogassi L. Neurons controlling voluntary vocalization in the macaque ventral premotor cortex. *PLoS One* 6: e26822, 2011.

Cromer J, Roy J, Miller E. Representation of multiple, independent categories in the primate prefrontal cortex. *Neuron* 66: 796–807, 2010.

Dawkins R, Krebs J. Arms race between and within species. *Proc Roy Soc B* 205: 489–511, 1979.

de la Mothe LA, Blumell S, Kajikawa Y, Hackett TA. Cortical connections of the auditory cortex in marmoset monkeys: core and medial belt regions. *J Comp Neurol* 496: 27–71, 2006.

de la Mothe LA, Blumell S, Kajikawa Y, Hackett TA. Cortical connections of the auditory cortex in marmoset monkeys: lateral belt and parabelt regions. *Anat Rec* 295: 800–821, 2012.

Deacon TW. *The Symbolic Species: The Co-evolution of Language and the Brain*. New York: W. W. Norton, 1997.

DiMattina C, Wang X. Virtual vocalization stimuli for investigating neural representations of species-specific vocalizations. *J Neurophysiol* 95: 1244–1262, 2006.

Duncan J. An adaptive coding model of neural function in prefrontal cortex. *Nat Rev Neurosci* 2: 820–829, 2001.

Egnor SER, Hauser MD. A paradox in the evolution of primate vocal learning. *Trends Neurosci* 27: 649–654, 2004.

Eliades S, Wang X. Neural correlates of the Lombard effect in primate auditory cortex. *J Neurosci* 32: 10737–10748, 2012.

Eliades SJ, Wang X. Chronic multi-electrode neural recording in free-roaming monkeys. *J Neurosci Meth* 172: 201–214, 2008a.

Eliades SJ, Wang X. Comparison of auditory-vocal interactions across multiple types of vocalizations in marmoset cortex. *J Neurophysiol* 109: 1638–1657, 2013.

Eliades SJ, Wang X. Neural substrates of vocalization feedback monitoring in primate auditory cortex. *Nature* 453: 1102–1106, 2008b.

Eliades SJ, Wang X. Sensory-motor interaction in the primate auditory cortex during self-initiated vocalizations. *J Neurophysiol* 89: 2185–2207, 2003.

Fitch WT. *The Evolution of Language*. New York: Cambridge University Press, 2010.

Fleagle JG. *Primate Adaptation and Evolution*. New York: Academic Press, 1998.

Fuster JM. *The Prefrontal Cortex*. New York: Academic Press, 2008.

Gabrieli JDE, Poldrack RA, Desmond JE. The role of left prefrontal cortex in language and memory. *Proc Natl Acad Scie USA* 95: 906–913, 1998.

Gemba H, Kyuhou S, Matsuzaki R, Amino Y. Cortical field potentials with audio-initiated vocalization in monkeys. *Neurosci Lett* 272: 49–52, 1999.

Ghazanfar AA, Maier JX, Hoffman KL, Logothetis NK. Multisensory integration of dynamic faces and voices in primate auditory cortex. *J Neurosci* 25: 5004–5012, 2005.

Gifford GW, MacLean KA, Hauser MD, Cohen YE. The neurophysiology of functionally meaningful categories: macaque ventrolateral prefrontal cortex plays a critical role in spontaneous categorization of species-specific vocalizations. *J Cog Neurosci* 17: 1471–1482, 2005.

Goodale MA, Milner AD. Separate visual pathways for perception and action. *Trends Neurosci* 15: 20–25, 1992.

Guilford T, Dawkins MS. Receiver psychology and the evolution of animal signals. *Anim Behav* 42: 1–14, 1991.

Hage SR, Nieder A. Single neurons in monkey prefrontal cortex encode volitional initiation of vocalizations. *Nat Commun* 4: 2409, 2013.

Hickok G, Poeppel D. The cortical organization of speech processing. *Nat Rev Neurosci* 8: 393–402, 2007.

Jurgens U. Neural pathways underlying vocal control. *Neurosci Biobehav Rev* 26: 235–258, 2002.

Jurgens U, Ploog D. Cerebral representation of vocalization in squirrel monkey. *Exp Brain Res* 10: 532–554, 1970.

Kajikawa Y, de la Mothe LA, Blumell S, Sterbing-D'Angelo SJ, D'Angelo W, Camalier CR, Hackett TA. Coding of FM sweep trains and twitter calls in area CM of marmoset auditory cortex. *Hear Res* 239: 107–125, 2008.

Kertesz A, McCabe P. Recovery patterns and prognosis in aphasia. *Brain* 100: 1–18, 1977.

Larson CR, Kistler MK. The relationship of periaqueductal gray neurons to vocalization and laryngeal EMG in the behaving monkey. *Exp Brain Res* 63: 596–606, 1986.

MacLean PD, Newman JD. Role of midline frontolimbic cortex in production of isolation call of squirrel monkeys. *Brain Res* 450: 111–123, 1988.

Mante V, Sussilo D, Shenoy K, Newsome WT. Context-dependent computation by recurrent dynamics in prefrontal cortex. *Nature* 503: 78–84, 2013.

Marler P. Representational vocal signals of primates. In: *Experimental Behavioral Ecology and Sociobiology*, edited by Ḧolldobler B, Lindauer M. Stuttgart, Germany: Gustav Fischer Verlag, 1985, p. 211–221.

Miller CT, Beck K, Meade B, Wang X. Antiphonal call timing in marmosets is behaviorally significant: interactive playback experiments. *J Comp Physiol A* 195: 783–789, 2009a.

Miller CT, Bee MA. Receiver psychology turns 20: is it time for a broader approach? *Anim Behav* 83: 331–343, 2012.

Miller CT, Dimauro A, Pistorio A, Hendry S, Wang X. Vocalization induced cFos expression in marmoset cortex. *Front Integr Neurosci* 4: -121–115, 2010a.

Miller CT, Eliades SJ, Wang X. Motor-planning for vocal production in common marmosets. *Anim Behav* 78: 1195–1203, 2009b.

Miller CT, Mandel K, Wang X. The communicative content of the common marmoset phee call during antiphonal calling. *Am J Primatol* 72: 974–980, 2010b.

Miller CT, Thomas AW. Individual recognition during bouts of antiphonal calling in common marmosets. *J Comp Physiol A* 198: 337–346, 2012.

Miller CT, Wang X. Sensory-motor interactions modulate a primate vocal behavior: antiphonal calling in common marmosets. *J Comp Physiol A* 192: 27–38, 2006.

Miller EK, Wallis JD. The prefrontal cortex and executive brain functions. In: *Fundamental Neuroscience* (4th Ed.), edited by Squire L, Berg D, Bloom FE, du Lac S, Ghosh A, Spitzer N. New York: Academic Press, 2012, p. 1069–1089.

Mitchell JF, Sundberg KA, Mitchell JH. Spatial attention decorelates intrinsic activity fluctuations in macaque area V4. *Neuron* 63: 879–888, 2009.

Morrill R, Thomas AW, Schiel N, Souto A, Miller CT. The effect of habitat acoustics on common marmoset vocal signal transmission. *Am J Primatol* 904–916, 2013.

Newman JD, Lindsley D. Single unit analysis of auditory processing in squirrel monkey frontal cortex. *Exp Brain Res* 25: 169–181, 1976.

Newman JD, Wollberg Z. Multiple coding of species-specific vocalizations in the auditory cortex of squirrel monkeys. *Brain Res* 54: 287–304, 1973a.

Newman JD, Wollberg Z. Responses of single neurons in the auditory cortex of squirrel monkeys to variants of a single call type. *Exp Neurol* 40: 821–824, 1973b.

Paxinos G, Watson CS, Petrides M, Rosa MGP, Tokuno H. *The Marmoset Brain Stereotaxic Coordinates*. New York: Academic Press, 2012.

Perrodin C, Kayser C, Logothetis NK, Petkov C. Voice cells in primate temporal lobe. *Curr Biol* 21: 1408–1415, 2011.

Petrides M, Cadoret G, Mackey S. Orofacial somatomotor responses in the macaque monkey homologue of Broca's area. *Nature* 435: 1235–1238, 2005.

Plakke B, Diltz M, Romanski LM. Coding of vocalizations by single neurons in ventrolateral prefrontal cortex. *Hear Res* 305: 135–143, 2013a.

Plakke B, NGC, Poremba A. Neural correlates of auditory recognition memory in primate lateral prefrontal cortex. *Neuroscience* 6: 62–76, 2013b.

Poeppel D, Hickok G. Towards a new functional anatomy of language. *Cognition* 92: 1–12, 2004.

Rauschecker JP. Ventral and dorsal streams in the evolution of speech and language. *Front Evol Neurosci* 4: 7, 2012.

Rauschecker JP, Scott SK. Maps and streams in the auditory cortex: nonhuman primates illuminate human speech processing. *Nat Neurosci* 12: 718–724, 2009.

Rauschecker JP, Tian B. Mechanisms and streams for processing of 'what' and 'where' in auditory cortex. *Proc Natl Acad Sci* 97: 18010–18015, 2000.

Recanzone GH, Cohen YE. Serial and parallel processing in the primate auditory cortex revisited. *Behav Brain Res* 206: 1–7, 2010.

Reser D, Burman KJ, Yu H, Chaplin TA, Richardson KE, Worthy KH, Rosa MGP. Contrasting patterns of circuit input to architectural subdivisions of the Area 8 complex: a retrograde tracing study in marmoset monkeys. *Cerebr Cortex* 8: 1901–1922, 2012.

Rigotti M, Barak O, Warden M, Wang X, Daw N, Miller E, Fusi S. The importance of mixed selectivity in complex cognitive tasks. *Nature* 497: 585–590, 2013.

Romanski LM, Averbeck BB. The primate cortical auditory system and neural representation of conspecific vocalizations. *Ann Rev Neurosci* 32: 315–346, 2009.

Romanski LM, Averbeck BB, Diltz M. Neural representation of vocalizations in the primate ventrolateral prefrontal cortex. *J Neurophysiol* 93: 734–747, 2005.

Romanski LM, Bates JF, Goldman-Rakic PS. Auditory belt and parabelt projections in the prefrontal cortex in the rhesus macaque. *J Comp Neurol* 403: 141–157, 1999a.

Romanski LM, Goldman-Rakic PS. An auditory domain in primate prefrontal. *Nat Neurosci* 5: 15–16, 2001.

Romanski LM, Tian B, Fritz J, Mishkin M, Goldman-Rakic PS, Rauschecker JP. Dual streams of auditory afferents target multiple domains in the primate prefrontal cortex. *Nat Neurosci* 2: 1131–1136, 1999b.

Roy S, Miller CT, Gottsch D, Wang X. Vocal control by the common marmoset in the presence of interfering noise. *J Exp Biol* 214: 3619–3629, 2011.

Schenker N, Desgoutes A, Semendeferi K. Neural connectivity and cortical substrates of cognition in hominoids. *J Hum Evol* 49: 547–569, 2005.

Semendeferi K, Armstrong E, Schleicher A, Zilles K, Van Hoesen G. Prefrontal cortex in humans and apes: a comparative study of area 10. *Am J Phys Anthropol* 114: 224–241, 2001.

Seyfarth RM, Cheney DL. Primate vocal communication. In: *Primate Neuroethology*, edited by Platt M, and Ghazanfar AA. New York: Oxford University Press, 2010, p. 84–97.

Simoes CS, Vianney PVR, Marcondes de Moura M, Freire MAM, Mello LE, Sameshima K, Araujo JF, Nicolelis MAL, Mello CV, Ribeiro S. Activation of frontal neocortical areas by vocal production in marmosets. *Front Integr Neurosci* 4: 1–12, 2010.

Simonyan K, Jurgens U. Afferent subcortical connections into the motor cortical larynx area in the rhesus monkey. *Neuroscience* 130: 119–131, 2005.

Stokes M, Kusunoki M, Sigala N, Nili H, Gaffan D, Duncan J. Dynamic coding for cognitive control in prefrontal cortex. *Neuron* 78: 364–375, 2013.

Takahashi D, Narayanan D, Ghazanfar AA. Coupled oscillator dynamics of vocal turn-taking in monkeys. *Curr Biol* 23: 2162–2168, 2013.

Tian B, Reser D, Durham A, Kustov A, Rauschecker JP. Functional specialization in rhesus monkey auditory cortex. *Science* 292: 290–293, 2001.

Ungerleider LG, Mishkin M. Two cortical visual systems. In: *Analysis of visual Behavior*, edited by Ingle DG, Goodale MA, Mansfield RJQ. Cambridge: MIT Press, 1982, p. 549–586.

Wang X, Merzenich MM, Beitel R, Schreiner CE. Representations of species-specific vocalizations in the primary auditory cortex of the common marmoset: temporal and spectral characteristics. *J Neurophysiol* 74: 2685–2706, 1995.

Winter P, Funkenstein HH. The effect of species-specific vocalizations on the discharge of auditory cortical cells in the awake squirrel monkey. *Exp Brain Res* 18: 489–504, 1973.

Wollberg Z, Newman JD. Auditory cortex of squirrel monkeys: response patterns of single cells to species-specific vocalizations. *Science* 175: 212–214, 1972.

Wong-Riley M. Changes in the visual system of monocularly sutured or enucleated cates demonstrable with cytochrome oxidase histology. *Brain Res* 1717: 11–28, 1979.

Zingale CM, Kowler E. Planning sequences of saccades. *Vision Res* 27: 1327–1341, 1987.