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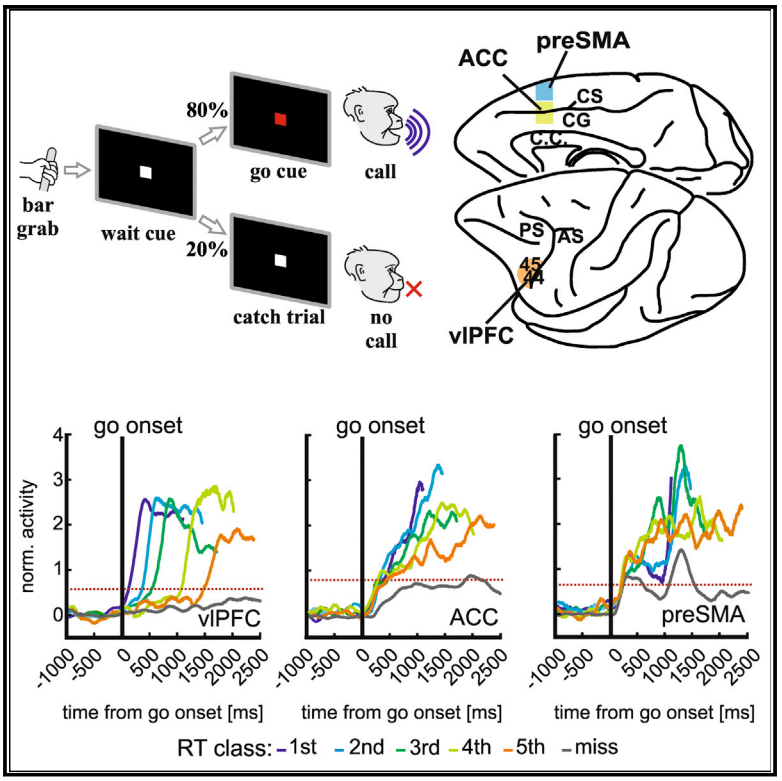
Functional Specialization of the Primate Frontal Lobe during Cognitive Control of Vocalizations

Graphical Abstract



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In Brief

Gavrilov et al. explored the roles of frontal lobe areas in initiating purposeful vocalizations. They recorded single-unit activity from the ventrolateral prefrontal cortex (vlPFC), the anterior cingulate cortex (ACC), and pre-supplementary motor area (preSMA) and found surprising differences between pre-vocal neural responses in the three brain areas.

Highlights

- Cellular activity recorded from frontal lobe in monkeys trained to call volitionally
- vlPFC encodes the decision to produce volitional calls
- ACC represents a motivational preparatory signal
- preSMA activity is consistent with a general motor priming signal

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Functional Specialization of the Primate Frontal Lobe during Cognitive Control of Vocalizations

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SUMMARY

Cognitive vocal control is indispensable for human language. Frontal lobe areas are involved in initiating purposeful vocalizations, but their functions remain elusive. We explored the respective roles of frontal lobe areas in initiating volitional vocalizations. Macaques were trained to vocalize in response to visual cues. Recordings from the ventrolateral prefrontal cortex (vlPFC), the anterior cingulate cortex (ACC), and the pre-supplementary motor area (preSMA) revealed single-neuron and population activity differences. Pre-vocal activity appeared first after the go cue in vlPFC, showing onset activity that was tightly linked to vocal reaction times. However, pre-vocal ACC onset activity was not indicative of call timing; instead, ramping activity reaching threshold values betrayed call onset. Neurons in preSMA showed weakest correlation with volitional call initiation and timing. These results suggest that vlPFC encodes the decision to produce volitional calls, whereas downstream ACC represents a motivational preparatory signal, followed by a general motor priming signal in preSMA.

INTRODUCTION

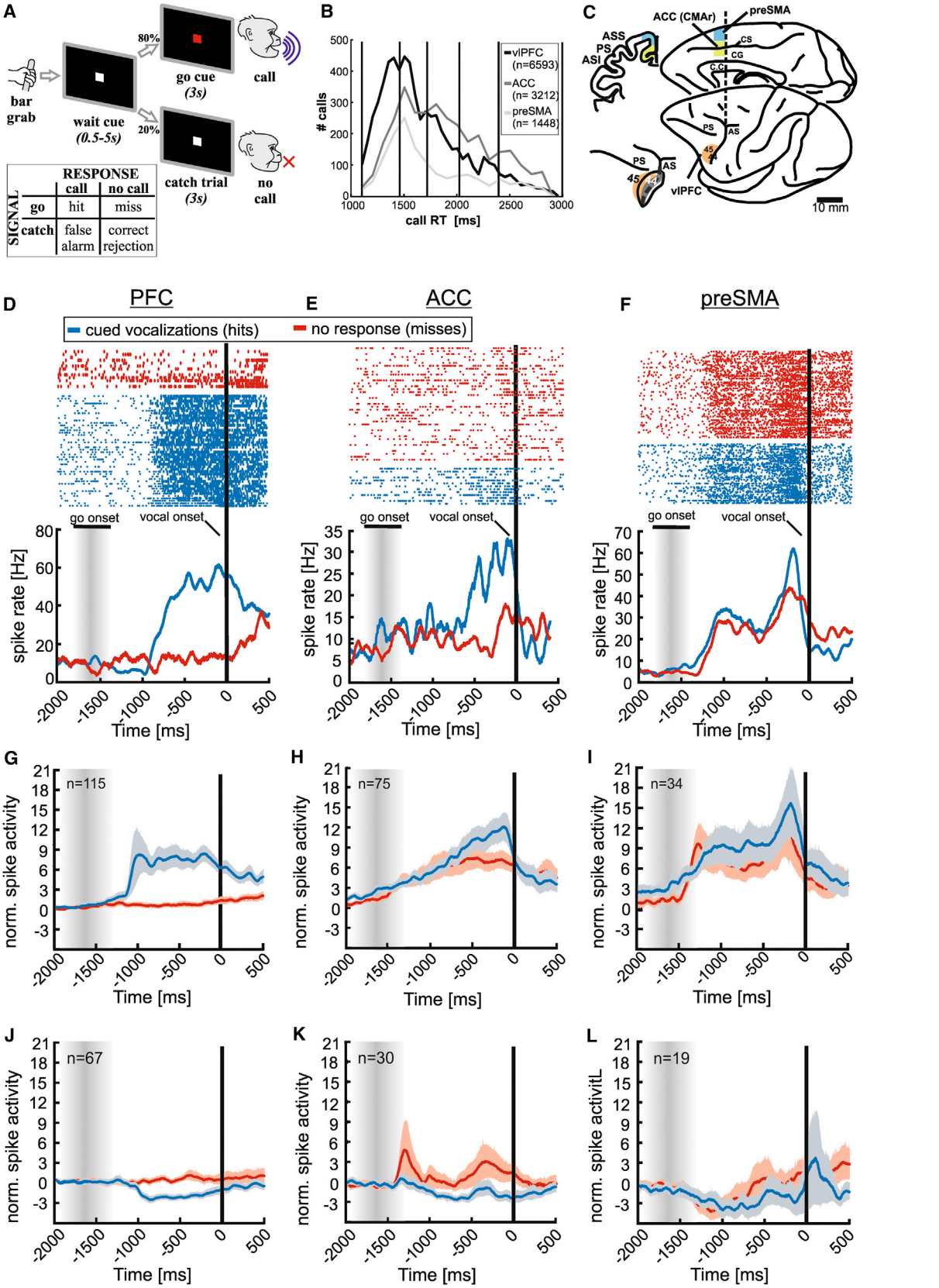
Humans are endowed with a sophisticated speech and language system that allows them to learn and use controlled vocalizations flexibly in linguistic symbol systems (Ghazanfar, 2008; Hammerschmidt and Fischer, 2008). This is in stark contrast to the stereotyped communication sounds in primates that are largely innate with only restricted flexibility (Takahashi et al., 2017; Gultekin and Hage, 2017) and that are usually uttered affectively (Jürgens, 2002; Ackermann et al., 2014). However, recent studies showed that monkeys can at least exert rudimentary cognitive control over their vocalizations (Coudé et al., 2011; Hage et al., 2013a). Monkeys can learn to call on command. This level of volitional control of vocalizations in nonhuman primates is an indispensable prerequisite for the evolution of the human speech system that requires a coupling of executive control structures to ancient vocal pattern-generating and limbic networks (Hage and Nieder, 2016). To date, the neuronal structures and brain processes enabling the initiation and planning of volitional vocalizations remain largely elusive.

A key articulation brain area endowing humans with vocal cognitive control is Broca's area (areas 44 and 45) in the inferior frontal gyrus of the granular ventrolateral prefrontal cortex (vlPFC). However, Broca's area is embedded in a larger frontal lobe speech network, including other structures, such as the anterior cingulate cortex (ACC) or the pre-supplementary motor area (preSMA) (Barris and Schuman, 1953; Nielsen and Jacobs, 1951; Nachev et al., 2008). How these structures are interacting within this network remains unclear.

In macaques, neuroanatomical studies identified a homolog of Broca's area in posterior parts of the vlPFC (Petrides and Pandya, 1999, 2002; Petrides et al., 2005). Moreover, recent electrophysiological experiments in behaving macaques reported activity related to cognitively controlled vocalizations in the monkey vlPFC (Hage and Nieder, 2013, 2015). The same area is also involved in audio-visual working memory (Romanski, 2012) and sequence processing (Wilson et al., 2015, 2017), indicating that the primate vlPFC is an evolutionary pre-adaptation for language functions in humans.

Whereas these data suggest that the monkey homolog of Broca's area is involved in the initiation of volitional calls, the precise role of the vlPFC remains unknown. Moreover, the vlPFC is, as in human speech production, likely only part of a larger frontal-lobe network responsible for controlled vocal production (Hage and Nieder, 2016; Loh et al., 2016). Lesion and electrical stimulation studies indicate that medial frontal lobe areas, specifically ACC and preSMA, might additionally be involved in controlling vocal output (Jürgens, 1976, 2002; Kirzinger and Jürgens, 1982; Sutton et al., 1985; Vogt and Barbas, 1988). In addition, recordings in monkeys suggested that the ACC is involved in call production (West and Larson, 1995) and the preSMA in voluntary movement selection (Shima and Tanji, 1998). Collectively, this evidence suggests the lateral PFC, the preSMA, and the ACC as parts of an interconnected frontal lobe vocalization network.

Here, we explored the behavioral relevance and relative contributions of these three frontal lobe areas in initiating cognitively controlled vocalizations. We recorded single-cell activity from the vlPFC, ACC, and preSMA while monkeys had to vocalize in response to the detection of an arbitrary visual cue. Our results show unexpected differences in response characteristics of



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vocalization-correlated neurons in the respective brain areas both at the level of responsive single neurons and unbiased neuronal populations.

RESULTS

While monkeys performed a visual detection task with vocalizations as cued responses (Figure 1A), we recorded the activity of single neurons from three frontal lobe areas: the vlPFC; the ACC; and the preSMA (Figure 1C). We were interested to determine the role of these areas in initiating goal-directed vocalizations. We first analyzed single-cell activity in the trial interval after go cue presentation but before call production. Next, we performed several population analyses to evaluate the temporal dynamics of the responses of vocalization-correlated neurons within the three frontal lobe areas relative to vocal output.

Responses Specific for Volitional Call Initiation

Neuronal activity that is related to the monkeys deciding and preparing to elicit a cued vocalization is expected to show different activation patterns in trials when the monkeys elicited the required vocalization to indicate a visual cue (hit trials) as opposed to trials also displaying a go cue but for which the monkeys missed to produce a vocalization (miss trials; Figure 1A). Indeed, many of the recorded neurons showed systematic changes in activity for hit trials compared to miss trials. The activity of three single neurons recorded from the PFC, the ACC, and the preSMA is shown in Figures 1D–1F. In this figure, neuronal activity is aligned relative to vocal onset (black horizontal bar in the dot raster and corresponding spike density histograms), with the go cue being presented on average about 1,500 ms before the call (see call reaction time [RT] distribution in Figure 1B). All three neurons show increased firing rates for hit trials compared to miss trials in the interval between go cue onset and call production. This indicates that these neurons are correlated with—or predictive of—goal-directed vocalizations.

We statistically tested the discharge rate differences in the interval between go cue onset and vocal onset in hit and miss trials for each single neuron. Neurons that showed a significant response difference in a 1,000-ms time window prior to vocal onset in hit trials compared to miss trials (Mann-Whitney U test; $p < 0.05$) were termed "vocalization-correlated neurons" (abbreviated "voc-neurons"). voc-neurons were most abundant in PFC with 33.4% (180/545) and in ACC with 34.7% (105/303) compared to preSMA with 21.8% (53/243). Roughly two-thirds of the voc-neurons increased activity during hit trials (excitatory voc-neurons), whereas the remaining one-third showed significantly diminished activity during hit trials (suppressive voc-neurons). The average normalized firing rates of all voc-neurons in the respective three recording areas are plotted in Figures 1G–1L. All excitatory voc-neurons from PFC, ACC, and preSMA are shown in Figures 1G–1I, whereas Figures 1J–1L display all suppressive voc-neurons.

Neurons playing a role in volitional call production are not only expected to show differential activity between hit and miss trials but also between cued vocalizations and spontaneous vocalizations unrelated to the task. Monkey T produced volitional and spontaneous coo calls. The spectrograms and the start frequencies in volitional and spontaneous calls were equal; small differences were only found for call duration and peak frequency (see Hage and Nieder, 2013 for details). Contrasting cued and spontaneous calls showed clear differences in firing rates prior to call production (Figure 2). Excitatory voc-neurons in all three brain areas greatly increased discharges in preparation of a cued vocalization but remained at baseline activity whenever the monkeys initiated a spontaneous, non-cued vocalization (Figures 2A–2D; Mann-Whitney U tests; $p < 0.05$). For suppressive voc-neurons, this analysis could only be done in the PFC, showing the equivalent but inverted effect: baseline activity was observed prior to spontaneous calls, whereas cued calls were preceded by remarkable suppression of neuronal responses (Figure 2B). The comparison of activity for hit versus miss trials combined with the comparison of discharges for cued versus spontaneous vocalizations collectively argue for distinct processes in the frontal lobe that are characteristic for volitional, goal-directed vocalizations.

Figure 1. Behavioral Protocol, Recording Sites, and Vocalization-Correlated Activity in Hit Trials Contrasted with Miss Trials

(A) Protocol of the go/no go vocalization task. Monkeys were trained to vocalize whenever a visual go cue (red or blue square) appeared. Inset: definition of trials according to signal detection theory is shown: vocalization to go stimulus, "hit"; no vocalization in response to go cue, "miss".

(B) Distribution of the monkeys' call reaction times during the recordings in the three frontal lobe areas vlPFC, ACC, and preSMA. Vertical lines indicate the temporal boundaries used to categories the RTs into five classes.

(C) Recording sites. Medial and lateral view of the monkey brain, showing the recording sites in vlPFC, preSMA, and ACC. AS, arcuate sulcus; ASi, inferior arcuate sulcus; ASs, superior arcuate sulcus; CG, cingulated gyrus; CS, cingulate sulcus; PS, principal sulcus.

(D–L) Pre-vocal neuronal responses aligned on vocal onset.

(D) Responses of an example neuron recorded in vlPFC show a significant increase of neuronal activity during trials with cued vocalizations (hit trials) in comparison with no response trials (misses). Upper panel shows the raster plot and the lower panel the corresponding spike density histogram averaged and smoothed with a Gaussian kernel for illustration. The vertical black line indicates the onset of vocalizations. Shaded area represents the distribution of go cue onsets.

(E and F) Responses of example neuron recorded in ACC (E) and preSMA (F) are shown.

(G–L) Averaged and normalized activity of vocalization-correlated neurons.

(G) Activity from PFC showing significant increase of pre-vocal responses during 1000ms before vocal onset.

(H) Activity from ACC showing significant increase of pre-vocal responses.

(I) Activity from preSMA showing significant increase of pre-vocal responses.

(J) Activity from PFC showing significant decrease of pre-vocal responses during 1000ms before vocal onset.

(K) Activity from ACC showing significant decrease of pre-vocal responses.

(L) Activity from preSMA showing significant decrease of pre-vocal responses. Shaded area around the curves depicts the SEM.

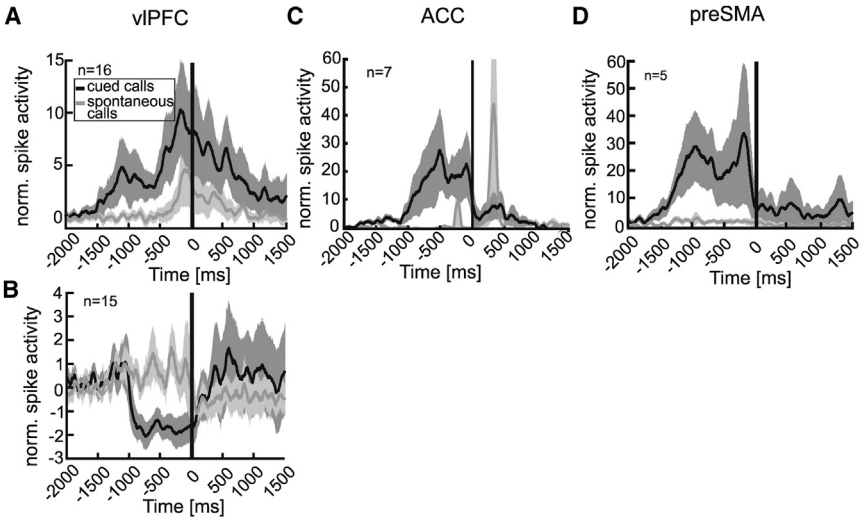

Figure 2. Pre-vocal Activity in Instructed Hit Trials Contrasted with Spontaneous Call Initiation

(A) Averaged and normalized activity of all vlPFC neurons recorded during cued as well as spontaneous vocalizations that showed increased activity prior to cued vocalizations (black vertical line, vocal onset; shaded area around the curves, SEM).

(B) Averaged and normalized activity of all vlPFC neurons that showed decreased activity prior to cued vocalization.

(C) Same as in (A) but for ACC neurons.

(D) Same as in (A) but for all preSMA neurons.

Correlating Neuronal Responses with Call RTs

The task design requires the monkeys to form a decision to call in response to the go-cue. On average, monkey T produced coo calls after a mean RT of 1.63 s, and monkey C uttered grunt calls after 1.89 s, resulting in a small difference of 0.26 s in RTs for the two call types and monkeys (Mann Whitney U test; $p < 0.01$). If voc-neurons play a role in the formation of the monkeys' decision to respond by vocalizing, they are expected to show systematic correlations with different RTs for calling. As witnessed by the distribution of RTs shown in Figure 1B, the monkeys sometimes responded quickly after go cue detection but other times more slowly. We exploited this trial-to-trial variation in RTs to explore whether and how the ramping activity of voc-neurons prior to cued vocalizations is related to call behavior.

First, we sorted the activity of single voc-neurons according to the call RTs of the monkeys in each trial into five classes, from fastest to longest RTs. The time course of activity for the five RT classes was then plotted relative to go cue onset (Figure 3, left column) and vocal onset (Figure 3, right column). Figure 3A displays the RT-sorted discharges for an exemplary vlPFC neuron. This neuron showed strong excitation whenever the monkey prepared a subsequent volitional call (colored traces) but remained at baseline activity for misses (gray traces). When the firing rate was aligned relative to the onset of the visual go cue, the onset of neuronal activity varied systematically with call RT, but not as a function of go cue presentation, resulting in a temporally staggered sequence of ramping activity (Figure 3A, left column). When the same neuronal responses were aligned relative to the onset of the vocalization, the firing rate functions were superimposed (Figure 3A, right column). Both the fast-rising slopes and the maximum activity plateaus for the five call RT classes were comparable. The time course of activity of this neuron precisely predicted the onset of the call after several hundred milliseconds. (It should be noted that the complex coordination of breathing, laryngeal, and mouth muscles for call production are known to require much longer than, for instance, eye or hand movements; this issue will be discussed later.) During miss trials, ramping activity was completely absent. All parameters indicate that this voc-neuron from the vlPFC was tightly linked to RTs.

Interestingly, the time course of vocalization-correlated activity for cells in the ACC and preSMA did not show such precise correlation with call initiation. Figure 3B shows a representative voc-neurons from ACC, again plotted relative to go cue onset (Figure 3B, left column) and vocal onset (Figure 3B, right column). Even though this neuron increased its firing rates between go cue onset and the start of the vocalization, no obvious temporal correlation with vocal onset was visible for this ACC voc-neuron.

Yet another response pattern was present for neurons in preSMA (Figure 3C). The depicted example neuron discharged reliably and temporally precise after go-cue onset (Figure 3C, left column), resulting in superimposed activity functions for all five vocal RT classes. Interestingly, the phasic response to go cue onset was also present for miss trials in which the monkey did not vocalize (gray trace). This miss trial response exhibited the same temporal onset and response magnitude as for hit trials. As a consequence of this response locked to the go cue, the same firing rates when aligned relative to vocal onset became temporally staggered.

To investigate this response pattern seen in representative single neurons, we analyzed the correlation of voc-neurons with call RTs. To that aim, the responses of all excitatory voc-neurons aligned to call onset were grouped into the five call RT classes (see Figure 3, right column), and the neuronal response latencies to the five call RT classes for each neuron were determined (based on objective threshold criteria; see Supplemental Experimental Procedures). (Due to their low response modulation, suppressive voc-neurons were not suitable to determine call-related neuronal latencies and excluded from this analysis.) Next, the neuronal latencies for each of the five call RT classes were plotted against increasing call RTs, and the slope from a regression line fitted to the data was derived. If the neuronal latencies are locked to vocal onset, the latencies should be equal for all call RTs, thus resulting in slopes around zero. However, if the neuronal latencies are not locked to vocal onset but to go cue onset, latencies will increase as a function of increasing RT classes, which results in positive regression slopes.

When we tested the distributions of resulting slopes per brain area, we found that the slopes of vlPFC neurons were distributed

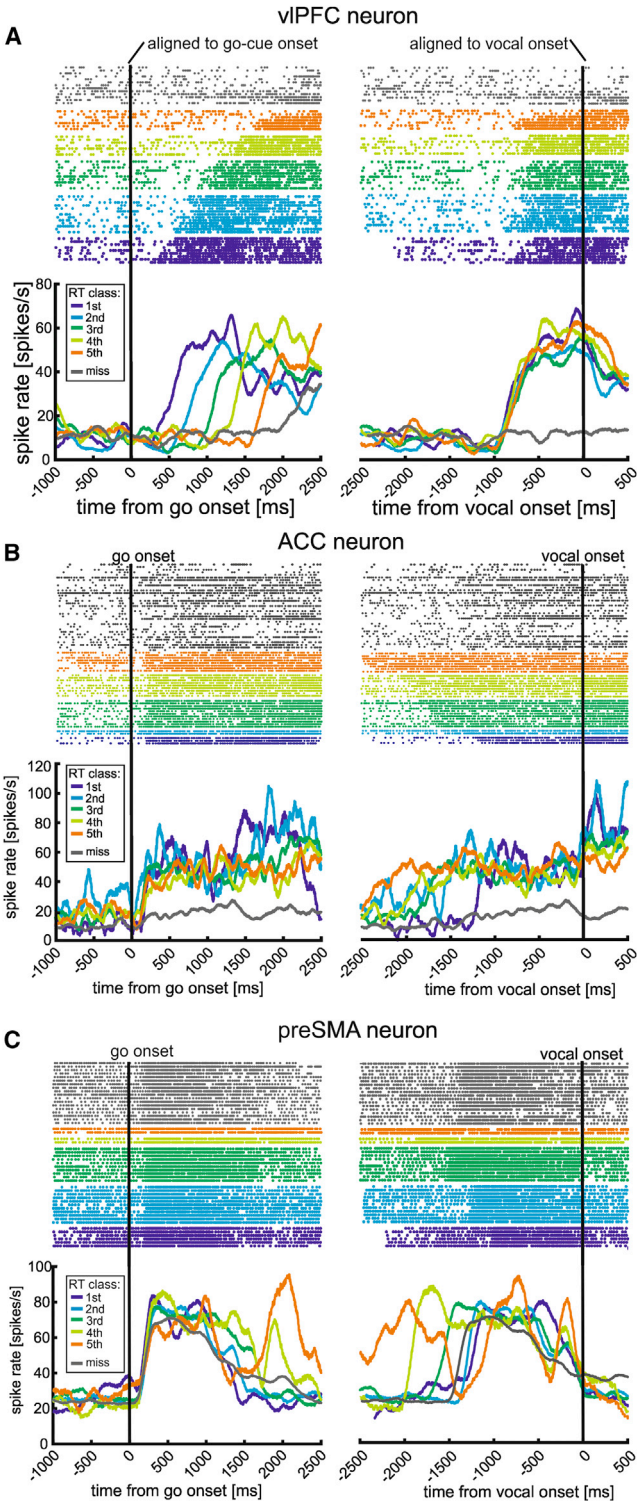

Figure 3. Temporal Correlation of Three Example Voc-Neurons with Call Reaction Time

(A) Time course of activity (hit trials) of a vlPFC example neuron showing significant increases of neuronal activity as a function of reaction times (RTs) (colored lines) in comparison to no response trials (misses, gray line). Upper panel shows the raster plot and the lower panel the corresponding spike density histogram averaged and smoothed with a Gaussian kernel for illustration. Left column shows the activity aligned on go cue onset. Right column represents the activity aligned on vocalization onset.

(B) Same as in (A) but for an exemplary ACC neuron.

(C) Same as in (A) but for an exemplary preSMA neuron.

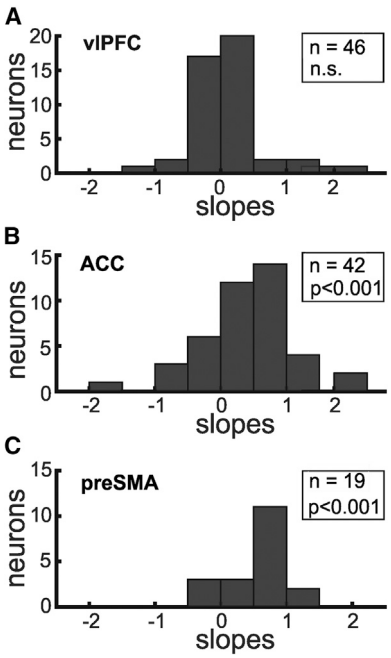

Figure 4. Distribution of Slopes Representing the Correlation of Neuronal Activity of Voc-Neurons with the Call RTs in the Corresponding Trials

(A) Distribution of slopes calculated in 46 excitatory vlPFC neurons.

(B) Distribution of slopes in 42 ACC neurons.

(C) Distribution of slopes calculated in 19 excitatory preSMA neurons (one-sample t test against a zero distribution; vlPFC $p = 0.18$; ACC $p < 0.001$; preSMA $p < 0.001$).

around zero (one-sample t test against a zero distribution; $p = 0.18$; Figure 4A). This indicates that the neuronal latencies of the population of vocalization-correlated PFC neurons were temporally linked to call onset, but not go cue onset. In contrast, both the latency distributions of ACC and preSMA neurons were significantly different from zero-slope distribution (one-sample t test against a zero distribution; $p < 0.001$; Figures 4B and 4C). In addition, both ACC and preSMA (mean slope of 0.44 and 0.60, respectively) had higher mean slopes compared to vlPFC (mean slope 0.13; $p < 0.05$; ANOVA corrected for multiple comparisons). This confirms that latencies in both ACC and preSMA areas were not locked to call onset but more to go cue onset.

To analyze the temporal and rate effects seen in single voc-neurons in more detail, we investigated the normalized and averaged time course of activation across the entire population of voc-neurons. The firing rates of all excitatory and suppressive voc-neurons were normalized relative to baseline activity (see Supplemental Experimental Procedures). The normalized activity of suppressive voc-neurons was rectified relative to

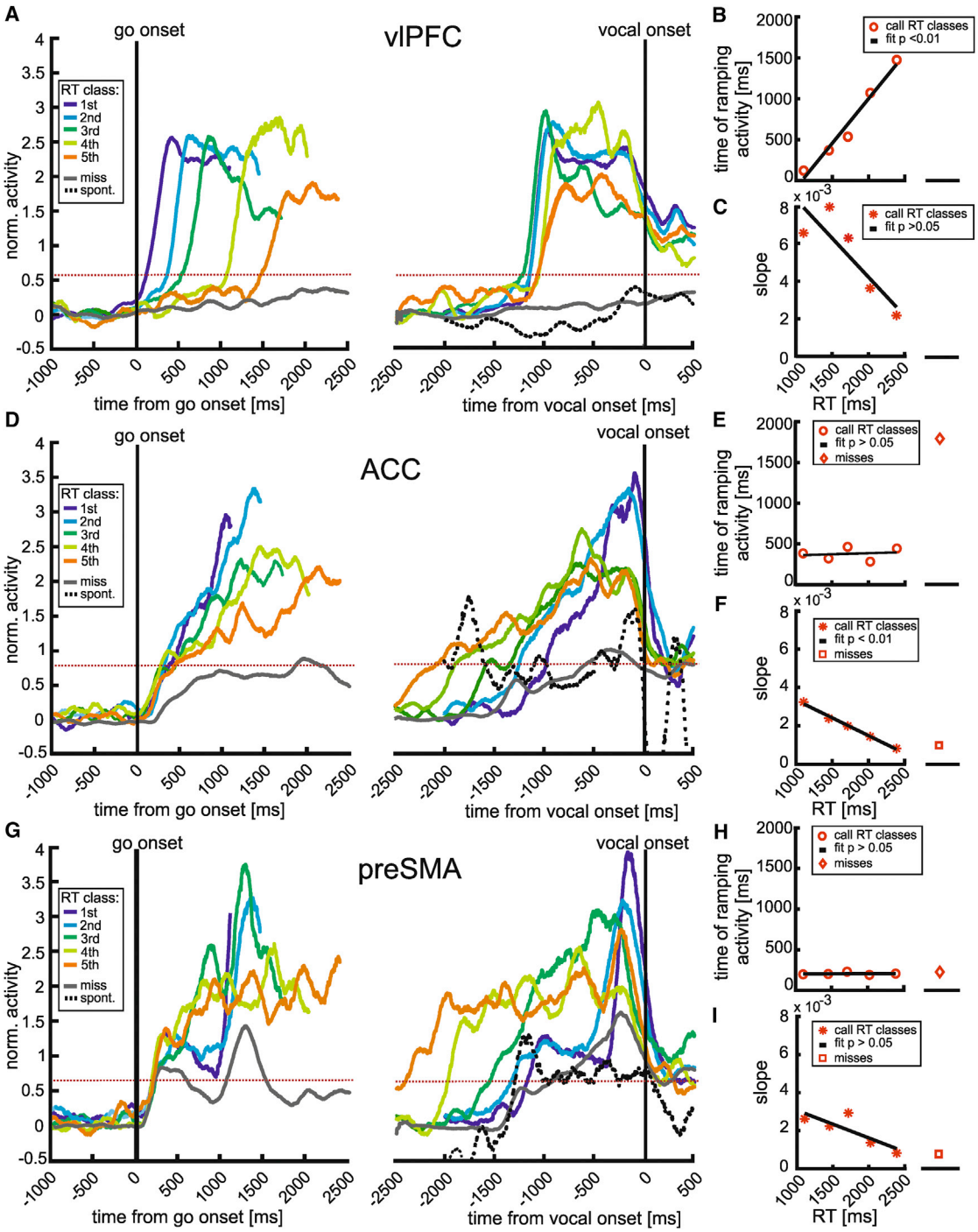

Figure 5. Temporal Correlation of the Populations of Voc-Neurons in Each of the Three Brain Areas with Call RT

(A) Averaged and normalized activity recorded in the vlPFC (n = 180), separated according to the call RT in the corresponding trial into five RT classes. Left column shows the time course of the activity in hit trials (colored lines) and miss trials (gray line) aligned on go cue onset (black vertical line). Right column shows the time course of activity in hit trials, miss trials, and during spontaneously (black, dotted line) uttered calls aligned on vocalization onset (black vertical line depicts vocal onset; red dotted line depicts threshold).

(B) Correlation of latency of ramping activity with the increasing call RTs in all vlPFC neurons. No values for the miss trials, because the activity in miss trials never reaches the threshold (red dotted line).

(C) Correlation of the slope steepness and the increasing call RTs in all vlPFC neurons. No values for the miss trials, because the activity in miss trials never reaches the threshold (red dotted line).

(D) Same as in (A) but for all ACC neurons (n = 105).

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baseline (mirrored at the baseline) so that negative deflections were translated into positive deflections of equal magnitude. This way, excitatory and suppressive voc-neurons could be averaged (in contrast to Figure 1, which shows these two groups of voc-neurons separately). Finally, the averaged normalized activity was again sorted according to the monkeys' call RTs into five increasing RT classes, and the time course of population activity in the three frontal areas for the five RT classes was plotted relative to go cue onset (Figures 5A, 5D, and 5G, left column) and vocal onset (Figures 5A, 5D, and 5G, right column).

In vlPFC, voc-neurons showed steeply rising activation flanks and systematic delays of ramping activation with call RTs when activity was aligned to go cue onset (Figure 5A, left column). However, when plotted according to vocal onset, the neuronal response functions became superimposed and showed almost identical temporal profiles. For all RTs, activity ramped steeply approximately one second prior to the vocalization to reach a plateau that mildly decayed (Figure 5A, right column). This activity was absent whenever the monkeys withheld the instructed calls during miss trials. Note that miss activity is only temporally precise when aligned relative to go cue onset (left part of the figure); for alignments relative to call onset (right part of the figure), the miss trial activity (that lack an RT by definition) is plotted relative to the average RT across all trials. The same effects are present if the averaged absolute firing rates of the neurons are plotted (Figure S1).

To quantify the relationship between neural activity and call initiation, we determined the time when the firing rate for all five RT classes started to rise (transition time from baseline activity to ramping activity). The neuronal ramping latency of the population was significantly correlated with call RTs ($p < 0.01$; Pearson's correlation; Figure 5B), indicating that the time point of ramping activity in vlPFC was tightly linked to call onset. Miss trial activity always remained at baseline and never reached the transition threshold.

In addition, we fitted the population ramping functions (from the start of the ramping activity to call onset) with linear functions and derived the slopes of the rising functions. As predicted from visual examination of the overlapping ramping functions (Figure 5A), the slopes of the five RT-grouped neuronal functions were not correlated with call RTs ($p > 0.05$; Pearson's correlation; Figure 5C). This means that not the slopes of the ramping activity of vlPFC neurons changed with call RT but the onset of the ramping activity.

When the data from the population of ACC voc-neurons were analyzed in the same way, a different picture emerged (Figures 5D–5F). ACC neurons showed ramping activity that increased for all RTs shortly after go cue onset (Figure 5D, left column). However, the steepness of the ramping activity declined systematically with increasing RTs (Figures 5E and 5F). Because the functions exhibiting shallower slopes for longer RTs also showed a longer duration, the climbing activity reached a similar firing rate value a few hundred milliseconds prior to call onset (Figure 5D). Also, in contrast to vlPFC, ramping activity for miss trials was observed in ACC, albeit with delayed onset and lower maximum firing rates. When we determined the onset and slopes of ACC ramping activity, ramping latency was not correlated with call RT and remained unchanged (Figure 5E; $p > 0.05$; Pearson's correlation). In other words, activity rose at about the same time after go cue onset, irrespective of how quickly the monkeys would call. In contrast to the vlPFC, however, the slopes of ACC activity significantly declined with call RT (Figure 5F; $p > 0.05$; Pearson's correlation).

Finally, the activity in preSMA was analyzed in the same way, showing yet another combination of effects. Right after go cue onset, activity steeply increased for all call RTs to reach an intermediate activation peak, followed by climbing activity until a second peak was reached just before call onset (Figure 5G). The phasic onset and sustained plateau is also evident if the averaged absolute firing rates of the neurons are plotted (Figure S1). Moreover, during miss trials, the overall temporal response profile was closely reminiscent of those of hit trials. Particularly right after go cue onset, the increase in firing rate for miss trials was virtually identical to hit trials. Only later in the trial, miss trials elicited lower firing rates but still a response profile that paralleled hit trials. Neither the onset of ramping activity (Figure 5H) nor its slopes were correlated with call RT (Figure 5I; both $p > 0.05$; Pearson's correlation).

Comparison of Vocalization-Correlated Cell Populations in vlPFC, ACC, and preSMA

The data so far suggest that the vlPFC has a privileged position among the three forebrain areas in initiating a volitional call. If this is true, we expect the vlPFC to host relatively many and intensely modulated voc-neurons that differentiated between hit and miss trials earlier than ACC or preSMA.

First, we focused on the population of voc-neurons, i.e., the selective neurons, in the respective brain areas. We found similarly high proportions of voc-neurons in PFC (33.4%) and ACC (34.7%) but significantly fewer neurons in preSMA (21.8%; χ^2 test; vlPFC-ACC: $p = 0.34$; vlPFC-preSMA: $p < 0.001$; ACC-preSMA: $p < 0.01$; Figure 6A).

Second, we compared the modulation indices between brain areas as a measure of coding strength of cued vocalizations. This analysis showed significantly higher modulation indices for voc-neurons in vlPFC (modulation index [MI] 0.47) compared to both ACC (MI: 0.34) and preSMA (MI: 0.30; Mann-Whitney U test; $p < 0.001$; Figure 6B). Despite the monkeys showing variable rates of hit and miss trials throughout the recording sessions (ranging from a minimum of 28% to a maximum of 92% per session; median 66%), the relative rates of hit or miss trials did not affect the strength of responses. The median MIs for the 50% lowest compared to 50% highest miss rates were as

(E) Correlation of latency of ramping activity with the increasing call RTs in all ACC neurons.
(F) Correlation of the slope steepness and the increasing call RTs in all ACC neurons.
(G) Same as in (A) but for all preSMA neurons (n = 105).
(H) Correlation of latency of ramping activity with the increasing call RTs in all preSMA neurons.
(I) Correlation of the slope steepness and the increasing call RTs in all preSMA neurons.

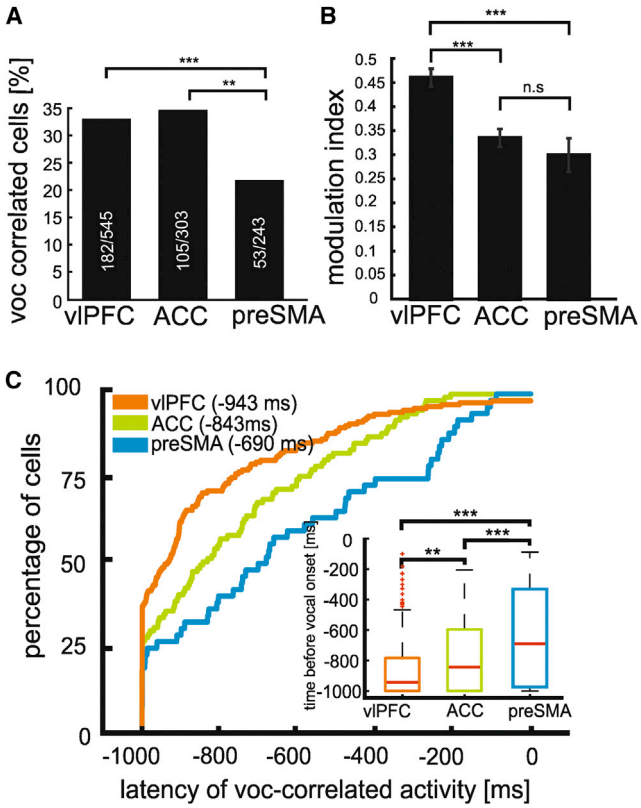

Figure 6. Comparison of Frequencies, Modulation Strengths, and Latencies of Voc-Neurons in the Three Brain Areas

(A) Proportions of voc-neurons. Bar plots show frequency distributions of voc-neurons in the vlPFC (vlPFC), the ACC, and the preSMA (***$p < 0.001$; **$p = 0.003$).

(B) Modulation index of voc-neurons. Bars show the median modulation index; error bar depicts SEM (***$p < 0.001$).

(C) Cumulative distribution of vocalization-correlated activity latencies for vlPFC, ACC, and preSMA neurons during 1,000 ms prior to vocalization onset. Latencies were measured by a sliding Kruskal-Wallis test.

follows: vlPFC: 0.52: 0.43; ACC: 0.34: 0.33; and preSMA: 0.30: 0.29 (Mann Whitney U test; all $p > 0.05$).

Third, we compared the latencies at which voc-neurons started to differentiate between hit and miss trials. The cumulative histogram for the latency of vocalization-correlated activity in Figure 6C shows that vlPFC neurons started to signal the upcoming cued vocalization earliest (mean latency: −943 ms), followed by ACC (−843 ms) and preSMA (−690 ms; sliding Kruskal-Wallis test; $p < 0.05$). (Note that some of the neurons were discriminating already prior to −1,000 ms, but the monkeys' variable call RT [minimum 1,100 ms] prevented us from analyzing earlier time periods.) These three parameters derived from the populations of voc-neurons suggest a hierarchy of processing stages between the three frontal lobe areas. The vlPFC shows the highest proportion of voc-neurons, with the highest coding strength and the fastest responses, suggesting that it adopts a prime position in initiating cognitively controlled vocalizations.

Next, we asked whether the effects we saw for voc-neurons were representative for all neurons in each area and at the level of the entire neuronal population. We therefore explored how whole neuronal populations of the three brain areas, irrespective of any selectivities or response preferences, encoded cued vocalizations in hit trials. We analyzed the coding capacity and dynamics of population responses as a whole by performing a multidimensional state space analysis (Yu et al., 2009; Gaussian process factor analysis [GPFA]) on equally sized pseudo-populations of neurons in vlPFC, ACC, and preSMA (156 vlPFC neurons, 158 ACC neurons, and 154 preSMA neurons). At each point in time, the activity of *n* recorded neurons can be defined by a point in *n*-dimensional space, with each dimension representing the activity of a single neuron. Dimensionality is effectively reduced to the three most informative dimensions using factor analysis (see Supplemental Experimental Procedures). Different trajectories are traversed for different neuronal states, i.e., they represent the encoding of hit and miss trials (Figures 7A–7C) in the respective brain areas. In other words, such trajectories reflect the instantaneous firing rate of the respective neuronal population as they evolve over time.

To evaluate the temporal evolution of population vocalization initiation activity in each brain area, we measured Euclidian distances between trial trajectories corresponding to hit and miss trials. This analysis was done for population responses either aligned to go cue onset (with variable vocal onset times; Figure 7D) or to vocal onset (with variable go onset times; Figure 7E). Both plots show largest inter-trajectory distances between hit and miss trials (i.e., strongest differentiation between hit and miss trials) for vlPFC, followed by ACC and finally preSMA with the weakest trajectory distances. Importantly, the increase of inter-trajectory distances occurred also earliest in vlPFC, followed by ACC and preSMA. This pattern of activation strength and time course in the whole populations of neurons in the respective brain areas was consistent with the findings based on only voc-neurons alone. The state-space analysis further corroborates a leading role of the vlPFC in volitional call initiation.

DISCUSSION

The first main conclusion from our study is that the vlPFC, the ACC, and the preSMA participate in volitional call initiation, albeit with different roles. In addition, the temporal dynamics of ramping activity in the time period between the instruction signal and the actual vocal output also suggest functional specializations in the respective brain areas: a deliberate decision signal emerging in the vlPFC; motivational coding in the ACC; and general preparatory motor signal in preSMA. It is worth mentioning that the recordings were obtained from juvenile monkeys progressing into adulthood. Both monkeys stopped volitional vocalizations once they reached full adulthood (Hage et al., 2013a). Our data were thus obtained from frontal lobe networks that may not have been fully mature.

From Motor Decision to Acoustic Output: Processing Times in the Vocal System

Vocalization-related activity in all three forebrain areas was measurable more than one second prior to the actual vocalization. This long preparatory activity of vocal neurons

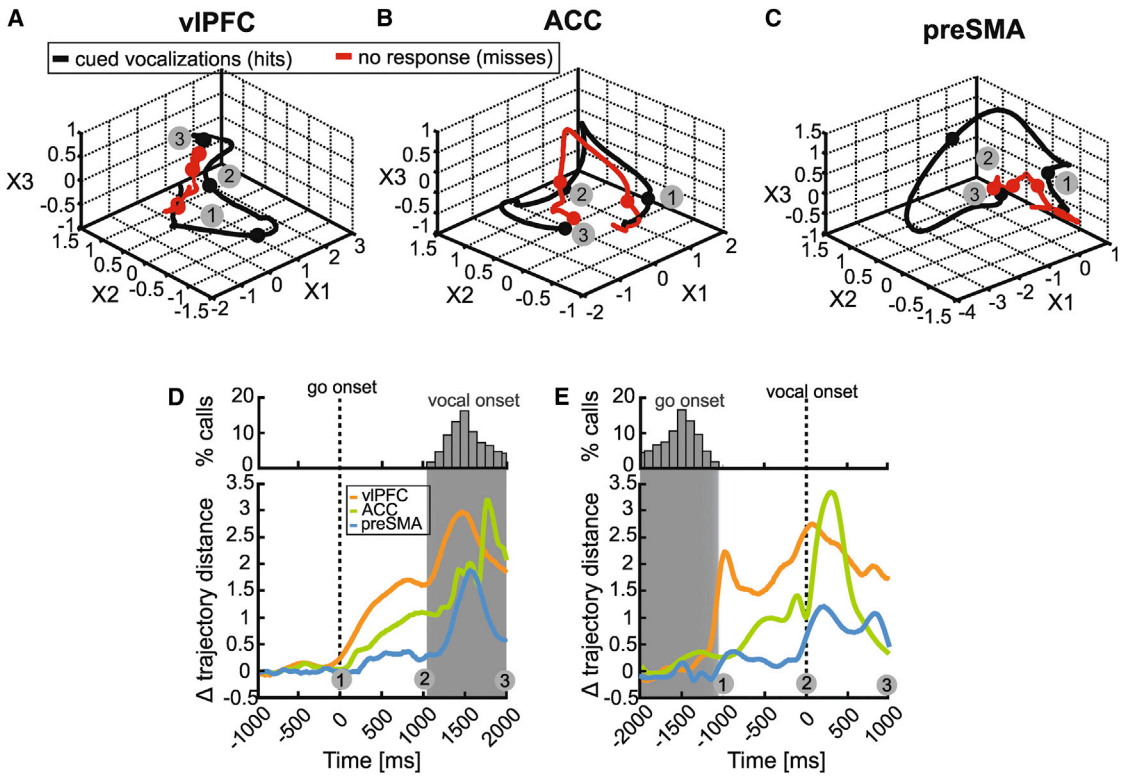

Figure 7. Temporal Evolution of Pre-vocal Activity in the Whole Populations of Neurons from vlPFC, ACC, and preSMA

(A–C) Gaussian process factor analysis delineating the state-space of neuronal population activity in the vlPFC (A; n = 156 neurons), preSMA (B; n = 154 neurons), and ACC (C; n = 158 neurons) over time for hit (black) and miss trials (red), respectively. Dots and numbers indicate beginning of the analysis window at −1, 000 ms (1), the vocal onset (2), and time point 1,000 ms after vocal onset (3).

(D and E) Inter-trajectory Euclidean distance over time, as a measure of neuronal selectivity for the initiation process of vocal output in vlPFC, ACC, and preSMA. (D) Activity aligned on go cue onset is shown. The vocal onset differs from trial to trial due to different response latencies. The distribution of all vocal onsets is depicted in the upper part of the figure. (E) Activity aligned on the vocal onset is shown. Upper part of the figure depicts the distribution of go cue onsets.

corresponded to long RTs of, on average, 1.5–2 s between the vocalization go cue and the call. Compared to the cued initiation of movements in other motor systems, such as hand or eye movements that only require monkeys a few tens to hundreds of milliseconds (Schultz et al., 1989; Nelson et al., 1990), neuronal latencies and behavioral RTs are unusually long in duration.

In contrast to arm or eye movements, for which only a few muscles are involved, vocal behavior is a complex motor pattern that requires the proper coordination of several functionally different muscle groups (Jürgens, 2002). During vocal output, muscles have to be controlled that are also used for vital stereotyped behaviors, such as respiration, swallowing, or mastication (Jürgens, 2002; Hage et al., 2013b). In rhesus macaques, the mean respiratory rate is 37 cycles per minute, resulting in a cycle length of 1,600 ms (Karel and Weston, 1946). Because calls require lung air pressure, the inhalation half cycle (ca. 800 ms) would be unsuited for vocalizing. Moreover, vocalizations require muscles to become activated well in advance of the onset of the acoustic signal. Therefore, the activity of muscles necessary for vocal behavior precedes the acoustic signal by up to 300 ms in macaque monkeys (West and Larson, 1993), such as the posterior cricoarytenoid (290 ms), cricothyroideus (240 ms), or intercostal (280 ms) muscles.

As a reflection of this advanced activation of muscle groups, preparatory neuronal latencies of premotor vocal neurons are already long in single neurons on subcortical level in macaque monkeys (Larson and Kistler, 1986; periaqueductal gray [PAG]: 200–900 ms) and squirrel monkeys (Düsterhöft et al., 2004; PAG: up to 300 ms). Single neurons on cortical level show pre-vocal activity with latencies up to 1,200 ms in ACC (West and Larson, 1995; dependent on call type) and up to 1,000 ms in the ventral premotor cortex (Coudé et al., 2011). The long premotor processing times required for vocal output are even reflected in the auditory cortex of marmosets, with significant pre-vocal suppression in population activity up to 750 ms before vocal onset, which has been interpreted to originate from cortical vocal production centers (Eliades and Wang, 2013).

More direct evidence for long-lasting preparatory activation of the vocal system comes from electrical stimulation. Minimal electrical stimulation latencies preceding vocal onset for cortical areas are as long as 2,900 ms in the posteromedial orbital cortex, 1,000 ms in the amygdala (Jürgens and Ploog, 1970), and with average latencies for the anterior

cingulate and supplementary motor area of squirrel monkeys of 2,200 ms (Jürgens, 1976). Downstream in the vocal motor hierarchy, minimal stimulation latencies decrease to 200 ms for the squirrel monkey PAG (Jürgens and Ploog, 1970). These stimulation latencies are in stark contrast to those reported for eye movements. Electrical stimulations within the frontal eye field elicited eye saccadic movements with latencies ranging from 20 to 60 ms (Bruce et al., 1985). Because of these processing delays, the deliberate decision to elicit a call is expected to occur more than a second earlier than the acoustic output in monkeys. This correlates with the longest onset of ramping activity as well as the longest pre-call latency of the vocalization-correlated activity in vlPFC.

Role of vlPFC in Volitional Call Initiation

The lateral PFC in general is implicated in volition and cognitive control of goal-directed actions (Miller et al., 2002; Eiselt and Nieder, 2013; Nieder, 2016). Recently, we demonstrated that neurons in the vlPFC also signal the preparation of volitional vocalizations (Hage and Nieder, 2013, 2015). Here, we showed that vlPFC adopts a privileged position among three frontal lobe areas with respect to the cognitive initiation of vocalizations. Relative to the ACC and the preSMA, which are also implicated in vocal behavior (Ackermann et al., 2014; Loh et al., 2016), vlPFC neurons showed the strongest and also earliest pre-vocal modulation and a high proportion of vocalization-correlated cells. The leading role of vlPFC was also confirmed for an unbiased population of vlPFC neurons irrespective of any selectivities or response preferences, showing that hit and miss trials were strongest and also earliest differentiated relative to ACC and preSMA.

Most importantly, vlPFC neurons showed a strong correlation between the timing of neuronal activity and the timing of vocal output. Irrespective of the call RTs, vlPFC neurons showed ramping onset around 1.2 s prior to the call (Figure 5A). These point to a direct involvement of the vlPFC in forming a decision signal for vocal motor output. Neurons in vlPFC showed a stereotyped ramp-to-threshold characteristic with equivalent slopes for all RTs. This ramping activity was dissimilar to perceptual decisions in which sensory parameters in noisy stimuli are thought to become integrated—or accumulated—over time, thus causing slopes of different steepness (Roitman and Shadlen, 2002). In contrast to a perceptual decision task, our task did not require accumulation of sensory evidence (the go cue was always salient). Therefore, the onset of this ramping activity was tightly linked to the monkeys RTs and precisely predicted vocal onset.

As an additional indicator of behavioral relevance, neuronal modulation was absent whenever the monkeys missed a cued vocalization or even when the monkeys vocalized spontaneously in between trials. The latter observation is particularly informative because the engagement of premotor, motor, and respiratory activity in spontaneous vocalizations is identical to volitional vocalizations.

In untrained marmosets, small changes (~1 Hz) in the population firing rate of frontal lobe neurons have been reported as a function of antiphonal conversations, suggesting a role of frontal lobe neurons in directed vocal communication (Nummela et al., 2017). However, whereas experimental control is difficult to achieve in spontaneously behaving animals, the current study targeted specific areas, sampled them densely, and could relate them to controlled experimental variables. The finding that vlPFC neurons showed the shortest latency of vocalization-correlated activity and was tightest linked to vocal RTs suggests the vlPFC as the first site where the decision to produce a vocalization in response to an instruction signal is formed.

The privileged cognitive-control position of the vlPFC is also supported by human studies. Direct cortical surface recordings in neurosurgical patients revealed that Broca's area is predominantly activated before the utterance of a speech signal but is silent during the corresponding articulation (Flinker et al., 2015), indicating that Broca's area is indirectly involved in coordinating speech initiation rather than in producing speech output directly (Lazar and Mohr, 2011; Dronkers and Baldo, 2010). Moreover, cooling of Broca's area in awake neurosurgical patients predominantly altered speech timing (Long et al., 2016), again indicating an involvement of Broca's area in speech coordination rather than in direct speech production. By contrast, focal cooling in speech motor cortex led to modulation of articulation, confirming the direct role of the speech motor cortex in articulation. The cognitive control signals we report in the vlPFC of monkeys suggest that the humble beginnings of speech control can already be witnessed in nonhuman primates (Hage and Nieder, 2016).

Role of ACC in Volitional Call Initiation

In the ACC, the rostral cingulate motor area (CMAr) located in the banks of the cingulate sulcus in the medial wall of the cortical hemispheres (Dum and Strick, 2002; Luppino et al., 1991; Matelli et al., 1991) is suggested to play a role in call production (West and Larson, 1995). The ACC receives direct or indirect inputs from the dorsolateral and orbital PFC, which are suitable to process cognitive aspects of motor control (Bates and Goldman-Rakic, 1993; Lu et al., 1994; Takada et al., 2004). In addition, afferents from the cingulate gyrus (areas 23 and 24) and from a variety of association and limbic areas implicate access of ACC to highly processed sensory and limbic information (Morecraft and Van Hoesen, 1998; Selemon and Goldman-Rakic, 1988; Vogt and Pandya, 1987). Besides efferents of the ACC to the primary motor cortex (Morecraft and Van Hoesen, 1992; Tokuno and Tanji, 1993), the ACC also targets premotor areas (Barbas and Pandya, 1987; Hatanaka et al., 2003; Simonyan, 2014), including the preSMA (Luppino et al., 1993; Wang et al., 2001).

The anatomical connection pattern suggests that the ACC operates downstream of the vlPFC in call production. Our recordings confirm a substantial involvement of the ACC in call preparation. We found an equally large proportion of voc-neurons in the ACC as in the vlPFC, albeit with significantly smaller modulation of pre-vocal activity. ACC neurons had significantly longer latencies of vocalization-correlated activities than vlPFC. Both of these findings were confirmed for the unbiased population of ACC neurons using state-space analysis. This delayed activation ACC would be consistent with this brain area receiving input from the vlPFC, as suggested by the mentioned anatomical connections.

The temporal activity profiles of ACC neurons contrasted those of vlPFC. The onset of the ramping activity began briefly after go cue onset and was uncorrelated with the monkeys' call RTs. Also unlike vlPFC, the onset of ACC activity was not predictive of call onset. Instead, the slopes of the ramping activity correlated with call RTs; the longer the RTs, the shallower the slopes. As a consequence, the time point of when the ramping activity reached a threshold was indicative of call RTs. This threshold activity was reached around the time when vlPFC neurons presumably signaled the decision to utter a cued vocalization (Figure 5B). Unlike vlPFC, ACC neurons were modulated after go cue onset during miss trials. Moreover, activity changes were observed prior to spontaneous vocalizations. Both of these findings suggest a more indirect involvement of ACC in volitional call initiation.

Collectively, these findings argue for a weaker involvement of the ACC in the initiation of volitional vocalizations. The temporal link of ACC activity with call RT was absent when the onset of the ramping activity was evaluated and only loosely present based on a similar threshold activity value reached around one second before call onset. Based on these findings, the ACC does not seem to represent a decision correlate for vocal production. Instead, we suspect the ACC to encode a supplementary preparatory signal. Based on the connections of the ACC with limbic structures, ACC ramping activity may reflect a motivational signal that needs to reach a threshold value for the monkey to activate the downstream motor neurons.

Our data in monkeys support a less cognitive but rather more affective and motivational role of the ACC in speech production (Jürgens, 2002; Paus, 2001). In monkeys, bilateral damage to the ACC prevents the initiation of calls in emotionally charged and social situations (Sutton et al., 1974; Aitken, 1981; MacLean and Newman, 1988). Lesion and electrical stimulation studies suggest an involvement of the ACC in controlling vocal output (Jürgens, 1976, 2002; Kirzinger and Jürgens, 1982; Sutton et al., 1985; Vogt and Barbas, 1988). Whether the ACC areas reported in different monkey species in these and our study are equivalent awaits further investigation.

In humans, bilateral lesions of the ACC produce akinetic mutism (Barris and Schuman, 1953; Nielsen and Jacobs, 1951). During recovery, the patient started to produce articulatory movements (i.e., whispering), possibly mediated by Broca's area, before monotonous speech was restored (Jürgens and von Cramon, 1982). Clinical studies also imply the ACC in the utterance of ill-controlled, non-verbal vocalizations, such as involuntary and stereotyped bursts of laughter ("gelastic seizures"; Wild et al., 2003; Kovac et al., 2009). Electrical stimulation of the rostral ACC (and the hypothalamus) elicited uncontrollable but natural-sounding laughter (Wild et al., 2003; Kuzniecky et al., 1997). Our recordings together with the clinical data from humans suggest that the vlPFC as central executive of a volitional articulation motor network controls affective vocalizations by influencing ACC as entry stage to a primary vocal motor network (Hage and Nieder, 2016).

Role of preSMA in Volitional Call Initiation

The preSMA (Matsuzaka et al., 1992), located in area 6 (premotor cortex) on the medial wall of the frontal cortex (Picard and Strick, 1996; Tanji, 1996), also receives direct afferents from the dorsolateral prefrontal cortex (Lu et al., 1994; Luppino et al., 1993), specifically from areas around the principal sulcus (areas 46d and 46v) and in the fundus of the inferior limb of the arcuate sulcus (area 44; Wang et al., 2005). Also, the ACC targets the preSMA (Luppino et al., 1993; Wang et al., 2001) as well as other premotor areas, but not primary motor cortex, allowing it to control motor commands.

Consistent with the anatomical connection patterns, neurons in preSMA showed vocalization-correlated activity prior to call onset. However, of the three areas investigated, the preSMA showed the weakest involvement in call initiation, both in terms of the number and modulation strength of voc-neurons. Following vlPFC and ACC, preSMA neurons became latest and weakest activated, a finding that was corroborated also at the unbiased population level.

The activity profiles of preSMA neurons contrasted those of vlPFC but also those of ACC. PreSMA neurons showed a phasic response shortly after go cue onset that was uncorrelated to call RT (Figure 5G). After this rapid onset activation, an elevated and plateau-like response period followed (see also Figure S1) with a brisk activation peak right before the vocalization. The onset of preSMA activity was not predictive of the call RTs but to some extent the slopes or threshold values. Almost the same temporal response profile, albeit at lower absolute activity values, was present during miss trials. Response modulation was also present prior to spontaneous vocalizations. These last two findings argue for a more indirect involvement of preSMA in volitional call initiation and potential sensitivity to general premotor factors.

Relative to vlPFC and ACC, preSMA activity seems to be remotest from—and least predictive of—a decision correlate. The enduring activation plateau shortly after go cue onset suggests some general readiness signal, potentially an elevated arousal level or a priming signal for a motor command. This interpretation would concur with the strong modulation of preSMA also during miss trials. Moreover, the relatively strong activation prior to the call suggests that preSMA activity leans more toward the output of a motor command rather than a cognitive initiation of vocalization. This interpretation would concur with human data. Brain imaging studies in humans have implicated the preSMA in motor task learning (Hikosaka et al., 1996). Moreover, preSMA is involved in word selection and production (Alario et al., 2006).

EXPERIMENTAL PROCEDURES

Surgical Procedures and Behavioral Protocol

All behavioral and physiological procedures were performed on two male macaque monkeys (*Macaca mulatta*) that both were five to seven years old during the recording sessions. All procedures were in accordance with the guidelines for animal experimentation and authorized by the national authorities (Regierungspräsidium Tübingen, Germany). Single-cell recordings were conducted in monkeys trained to vocalize in response to an arbitrary visual cue in a go/no go detection task as described earlier (Hage et al., 2013a; Hage and Nieder, 2013). Single-cell recording was performed in three brain areas: the preSMA; the CMAr of the ACC; and the vlPFC (area 44 and 45) in the behaving monkeys.

Data Analysis

We analyzed pre-vocal activity in a 1,000-ms window before vocalization and compared firing rates within this time interval between trials with cued

vocalizations (hit trials) and silent trials in which the monkey withheld vocalizations (miss trials). In addition, pre-vocal neuronal activity was compared between volitional "coo" calls uttered during cued vocalization trials and spontaneous coos vocalized. Normalized activity was calculated by subtracting the mean neuronal baseline activity from the neuronal responses and dividing the outcome by the SD of the baseline activity.

To determine the latency of vocalization-correlated activity, we computed a sliding Kruskal-Wallis test in 100-ms windows that were slid in 20-ms steps. To quantify the coding strength of the vocalization initiation, we calculated a MI for each voc-neurons.

To study how the recorded neuronal populations as a whole dynamically encode the initiation of volitional call production, we represent population activity as trajectories in a multidimensional state space. To that aim, we performed a GPFA to extract smooth, low-dimensional neuronal trajectories from the high-dimensional noisy spiking activity.

To investigate the correlation of the pre-vocal activity and the call RTs in populations of voc-neurons, we aligned the activity of all excitatory neurons on vocal onset (separated in five classes) and determined the neuronal latency for each neuron and call RT class.

SUPPLEMENTAL INFORMATION

The Supplemental Information includes Supplemental Experimental Procedures and one figure and can be found with this article online at https://doi.org/10.1016/j.celrep.2017.10.107.

AUTHOR CONTRIBUTIONS

N.G. and S.R.H. performed the experiments; S.R.H. and A.N. designed the study; N.G. and A.N. analyzed the data; and N.G., S.R.H., and A.N. interpreted the data and wrote the manuscript.

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REFERENCES

Ackermann, H., Hage, S.R., and Ziegler, W. (2014). Brain mechanisms of acoustic communication in humans and nonhuman primates: an evolutionary perspective. Behav. Brain Sci. *37*, 529–546.

Aitken, P.G. (1981). Cortical control of conditioned and spontaneous vocal behavior in rhesus monkeys. Brain Lang. *13*, 171–184.

Alario, F.X., Chainay, H., Lehericy, S., and Cohen, L. (2006). The role of the supplementary motor area (SMA) in word production. Brain Res. *1076*, 129–143.

Barbas, H., and Pandya, D.N. (1987). Architecture and frontal cortical connections of the premotor cortex (area 6) in the rhesus monkey. J. Comp. Neurol. *256*, 211–228.

Barris, R.W., and Schuman, H.R. (1953). [Bilateral anterior cingulate gyrus lesions; syndrome of the anterior cingulate gyri]. Neurology *3*, 44–52.

Bates, J.F., and Goldman-Rakic, P.S. (1993). Prefrontal connections of medial motor areas in the rhesus monkey. J. Comp. Neurol. *336*, 211–228.

Bruce, C.J., Goldberg, M.E., Bushnell, M.C., and Stanton, G.B. (1985). Primate frontal eye fields. II. Physiological and anatomical correlates of electrically evoked eye movements. J. Neurophysiol. *54*, 714–734.

Coudé, G., Ferrari, P.F., Rodà, F., Maranesi, M., Borelli, E., Veroni, V., Monti, F., Rozzi, S., and Fogassi, L. (2011). Neurons controlling voluntary vocalization in the macaque ventral premotor cortex. PLoS ONE *6*, e26822.

Dronkers, N.F., and Baldo, J.V. (2010). Broca's area. In The Cambridge Encyclopedia of the Language Sciences, P.C. Hogan, ed. (Cambridge University Press), pp. 139–142.

Dum, R.P., and Strick, P.L. (2002). Motor areas in the frontal lobe of the primate. Physiol. Behav. *77*, 677–682.

Düsterhöft, F., Häusler, U., and Jürgens, U. (2004). Neuronal activity in the periaqueductal gray and bordering structures during vocal communication in the squirrel monkey. Neuroscience *123*, 53–60.

Eiselt, A.K., and Nieder, A. (2013). Representation of abstract quantitative rules applied to spatial and numerical magnitudes in primate prefrontal cortex. J. Neurosci. *33*, 7526–7534.

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

Flinker, A., Korzeniewska, A., Shestyuk, A.Y., Franaszczuk, P.J., Dronkers, N.F., Knight, R.T., and Crone, N.E. (2015). Redefining the role of Broca's area in speech. Proc. Natl. Acad. Sci. USA *112*, 2871–2875.

Ghazanfar, A.A. (2008). Language evolution: neural differences that make a difference. Nat. Neurosci. *11*, 382–384.

Gultekin, Y.B., and Hage, S.R. (2017). Limiting parental feedback disrupts vocal development in marmoset monkeys. Nat. Commun. *8*, 14046.

Hage, S.R., and Nieder, A. (2013). Single neurons in monkey prefrontal cortex encode volitional initiation of vocalizations. Nat. Commun. *4*, 2409.

Hage, S.R., and Nieder, A. (2015). Audio-vocal interaction in single neurons of the monkey ventrolateral prefrontal cortex. J. Neurosci. *35*, 7030–7040.

Hage, S.R., and Nieder, A. (2016). Dual neural network model for the evolution of speech and language. Trends Neurosci. *39*, 813–829.

Hage, S.R., Gavrilov, N., and Nieder, A. (2013a). Cognitive control of distinct vocalizations in rhesus monkeys. J. Cogn. Neurosci. *25*, 1692–1701.

Hage, S.R., Gavrilov, N., Salomon, F., and Stein, A.M. (2013b). Temporal vocal features suggest different call-pattern generating mechanisms in mice and bats. BMC Neurosci. *14*, 99.

Hammerschmidt, K., and Fischer, J. (2008). Constraints in primate vocal production. In Evolution of Communicative Flexibility: Complexity, Creativity, and Adaptability in Human and Animal Communication, D.K. Oller and U. Griebel, eds. (Cambridge: MIT Press), pp. 93–121.

Hatanaka, N., Tokuno, H., Hamada, I., Inase, M., Ito, Y., Imanishi, M., Hasegawa, N., Akazawa, T., Nambu, A., and Takada, M. (2003). Thalamocortical and intracortical connections of monkey cingulate motor areas. J. Comp. Neurol. *462*, 121–138.

Hikosaka, O., Sakai, K., Miyauchi, S., Takino, R., Sasaki, Y., and Pütz, B. (1996). Activation of human presupplementary motor area in learning of sequential procedures: a functional MRI study. J. Neurophysiol. *76*, 617–621.

Jürgens, U. (1976). Reinforcing concomitants of electrically elicited vocalizations. Exp. Brain Res. *26*, 203–214.

Jürgens, U. (2002). Neural pathways underlying vocal control. Neurosci. Biobehav. Rev. *26*, 235–258.

Jürgens, U., and Ploog, D. (1970). Cerebral representation of vocalization in the squirrel monkey. Exp. Brain Res. *10*, 532–554.

Jürgens, U., and von Cramon, D. (1982). On the role of the anterior cingulate cortex in phonation: a case report. Brain Lang. *15*, 234–248.

Karel, L., and Weston, R.E. (1946). Respiration in Macaca mulatta (Rhesus monkey). Proc. Soc. Exp. Biol. Med. *61*, 291–296.

Kirzinger, A., and Jürgens, U. (1982). Cortical lesion effects and vocalization in the squirrel monkey. Brain Res. *233*, 299–315.

Kovac, S., Deppe, M., Mohammadi, S., Schiffbauer, H., Schwindt, W., Möddel, G., Dogan, M., and Evers, S. (2009). Gelastic seizures: a case of lateral frontal lobe epilepsy and review of the literature. Epilepsy Behav. *15*, 249–253.

Kuzniecky, R., Guthrie, B., Mountz, J., Bebin, M., Faught, E., Gilliam, F., and Liu, H.G. (1997). Intrinsic epileptogenesis of hypothalamic hamartomas in gelastic epilepsy. Ann. Neurol. *42*, 60–67.

Larson, C.R., and Kistler, M.K. (1986). The relationship of periaqueductal gray neurons to vocalization and laryngeal EMG in the behaving monkey. Exp. Brain Res. *63*, 596–606.

Lazar, R.M., and Mohr, J.P. (2011). Revisiting the contributions of Paul Broca to the study of aphasia. Neuropsychol. Rev. *21*, 236–239.

Loh, K.K., Petrides, M., Hopkins, W.D., Procyk, E., and Amiez, C. (2016). Cognitive control of vocalizations in the primate ventrolateral-dorsomedial frontal (VLF-DMF) brain network. Neurosci. Biobehav. Rev., S0149-7634(16) 30366-9.

Long, M.A., Katlowitz, K.A., Svirsky, M.A., Clary, R.C., Byun, T.M., Majaj, N., Oya, H., Howard, M.A., 3rd, and Greenlee, J.D.W. (2016). Functional segregation of cortical regions underlying speech timing and articulation. Neuron *89*, 1187–1193.

Lu, M.T., Preston, J.B., and Strick, P.L. (1994). Interconnections between the prefrontal cortex and the premotor areas in the frontal lobe. J. Comp. Neurol. *341*, 375–392.

Luppino, G., Matelli, M., Camarda, R.M., Gallese, V., and Rizzolatti, G. (1991). Multiple representations of body movements in mesial area 6 and the adjacent cingulate cortex: an intracortical microstimulation study in the macaque monkey. J. Comp. Neurol. *311*, 463–482.

Luppino, G., Matelli, M., Camarda, R., and Rizzolatti, G. (1993). Corticocortical connections of area F3 (SMA-proper) and area F6 (pre-SMA) in the macaque monkey. J. Comp. Neurol. *338*, 114–140.

MacLean, P.D., and Newman, J.D. (1988). Role of midline frontolimbic cortex in production of the isolation call of squirrel monkeys. Brain Res. *450*, 111–123.

Matelli, M., Luppino, G., and Rizzolatti, G. (1991). Architecture of superior and mesial area 6 and the adjacent cingulate cortex in the macaque monkey. J. Comp. Neurol. *311*, 445–462.

Matsuzaka, Y., Aizawa, H., and Tanji, J. (1992). A motor area rostral to the supplementary motor area (presupplementary motor area) in the monkey: neuronal activity during a learned motor task. J. Neurophysiol. *68*, 653–662.

Miller, E.K., Freedman, D.J., and Wallis, J.D. (2002). The prefrontal cortex: categories, concepts and cognition. Philos. Trans. R. Soc. Lond. B Biol. Sci. *357*, 1123–1136.

Morecraft, R.J., and Van Hoesen, G.W. (1992). Cingulate input to the primary and supplementary motor cortices in the rhesus monkey: evidence for somatotopy in areas 24c and 23c. J. Comp. Neurol. *322*, 471–489.

Morecraft, R.J., and Van Hoesen, G.W. (1998). Convergence of limbic input to the cingulate motor cortex in the rhesus monkey. Brain Res. Bull. *45*, 209–232.

Nachev, P., Kennard, C., and Husain, M. (2008). Functional role of the supplementary and pre-supplementary motor areas. Nat. Rev. Neurosci. *9*, 856–869.

Nelson, R.J., McCandlish, C.A., and Douglas, V.D. (1990). Reaction times for hand movements made in response to visual versus vibratory cues. Somatosens. Mot. Res. *7*, 337–352.

Nieder, A. (2016). The neuronal code for number. Nat. Rev. Neurosci. *17*, 366–382.

Nielsen, J.M., and Jacobs, L.L. (1951). Bilateral lesions of the anterior cingulate gyri; report of case. Bull. Los Angel. Neuro. Soc. *16*, 231–234.

Nummela, S.U., Jovanovic, V., de la Mothe, L., and Miller, C.T. (2017). Social context-dependent activity in marmoset frontal cortex populations during natural conversations. J. Neurosci. *37*, 7036–7047.

Paus, T. (2001). Primate anterior cingulate cortex: where motor control, drive and cognition interface. Nat. Rev. Neurosci. *2*, 417–424.

Petrides, M., and Pandya, D.N. (1999). Dorsolateral prefrontal cortex: comparative cytoarchitectonic analysis in the human and the macaque brain and corticocortical connection patterns. Eur. J. Neurosci. *11*, 1011–1036.

Petrides, M., and Pandya, D.N. (2002). Comparative cytoarchitectonic analysis of the human and the macaque ventrolateral prefrontal cortex and corticocortical connection patterns in the monkey. Eur. J. Neurosci. *16*, 291–310.

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

Picard, N., and Strick, P.L. (1996). Motor areas of the medial wall: a review of their location and functional activation. Cereb. Cortex *6*, 342–353.

Roitman, J.D., and Shadlen, M.N. (2002). Response of neurons in the lateral intraparietal area during a combined visual discrimination reaction time task. J. Neurosci. *22*, 9475–9489.

Romanski, L.M. (2012). Integration of faces and vocalizations in ventral prefrontal cortex: implications for the evolution of audiovisual speech. Proc. Natl. Acad. Sci. USA *109* (*Suppl 1*), 10717–10724.

Schultz, W., Romo, R., Scarnati, E., Sundström, E., Jonsson, G., and Studer, A. (1989). Saccadic reaction times, eye-arm coordination and spontaneous eye movements in normal and MPTP-treated monkeys. Exp. Brain Res. *78*, 253–267.

Selemon, L.D., and Goldman-Rakic, P.S. (1988). Common cortical and subcortical targets of the dorsolateral prefrontal and posterior parietal cortices in the rhesus monkey: evidence for a distributed neural network subserving spatially guided behavior. J. Neurosci. *8*, 4049–4068.

Shima, K., and Tanji, J. (1998). Role for cingulate motor area cells in voluntary movement selection based on reward. Science *282*, 1335–1338.

Simonyan, K. (2014). The laryngeal motor cortex: its organization and connectivity. Curr. Opin. Neurobiol. *28*, 15–21.

Sutton, D., Larson, C., and Lindeman, R.C. (1974). Neocortical and limbic lesion effects on primate phonation. Brain Res. *71*, 61–75.

Sutton, D., Trachy, R.E., and Lindeman, R.C. (1985). Discriminative phonation in macaques: effects of anterior mesial cortex damage. Exp. Brain Res. *59*, 410–413.

Takada, M., Nambu, A., Hatanaka, N., Tachibana, Y., Miyachi, S., Taira, M., and Inase, M. (2004). Organization of prefrontal outflow toward frontal motor-related areas in macaque monkeys. Eur. J. Neurosci. *19*, 3328–3342.

Takahashi, D.Y., Liao, D.A., and Ghazanfar, A.A. (2017). Vocal learning via social reinforcement by infant marmoset monkeys. Curr. Biol. *27*, 1844–1852.e6.

Tanji, J. (1996). New concepts of the supplementary motor area. Curr. Opin. Neurobiol. *6*, 782–787.

Tokuno, H., and Tanji, J. (1993). Input organization of distal and proximal forelimb areas in the monkey primary motor cortex: a retrograde double labeling study. J. Comp. Neurol. *333*, 199–209.

Vogt, B.A., and Barbas, H. (1988). Structure and connections of the cingulate vocalization region in the rhesus monkey. In The Physiological Control of Mammalian Vocalization, J.D. Newman, ed. (Plenum Press), pp. 203–225.

Vogt, B.A., and Pandya, D.N. (1987). Cingulate cortex of the rhesus monkey: II. Cortical afferents. J. Comp. Neurol. *262*, 271–289.

Wang, Y., Shima, K., Sawamura, H., and Tanji, J. (2001). Spatial distribution of cingulate cells projecting to the primary, supplementary, and pre-supplementary motor areas: a retrograde multiple labeling study in the macaque monkey. Neurosci. Res. *39*, 39–49.

Wang, Y., Isoda, M., Matsuzaka, Y., Shima, K., and Tanji, J. (2005). Prefrontal cortical cells projecting to the supplementary eye field and presupplementary motor area in the monkey. Neurosci. Res. *53*, 1–7.

West, R., and Larson, C.R. (1993). Laryngeal and respiratory activity during vocalization in macaque monkeys. J. Voice *7*, 54–68.

West, R.A., and Larson, C.R. (1995). Neurons of the anterior mesial cortex related to faciovocal activity in the awake monkey. J. Neurophysiol. *74*, 1856–1869.

Wild, B., Rodden, F.A., Grodd, W., and Ruch, W. (2003). Neural correlates of laughter and humour. Brain *126*, 2121–2138.

Wilson, B., Kikuchi, Y., Sun, L., Hunter, D., Dick, F., Smith, K., Thiele, A., Griffiths, T.D., Marslen-Wilson, W.D., and Petkov, C.I. (2015). Auditory sequence processing reveals evolutionarily conserved regions of frontal cortex in macaques and humans. Nat. Commun. *6*, 8901.

Wilson, B., Marslen-Wilson, W.D., and Petkov, C.I. (2017). Conserved sequence processing in primate frontal cortex. Trends Neurosci. *40*, 72–82.

Yu, B.M., Cunningham, J.P., Santhanam, G., Ryu, S.I., Shenoy, K.V., and Sahani, M. (2009). Gaussian-process factor analysis for low-dimensional single-trial analysis of neural population activity. J. Neurophysiol. *102*, 614–635.