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A primary role for nucleus accumbens and related limbic network in vocal tics

--Manuscript Draft--

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Abstract:	Inappropriate vocal expressions - e.g., vocal tics in Tourette syndrome - severely impact quality of life. Neural mechanisms underlying vocal tics remain unexplored because of no established animal model representing the condition. We report unilateral disinhibition of the nucleus accumbens (NAc) generates vocal tics in monkeys. Whole-brain PET imaging identified prominent, bilateral limbic cortico-subcortical activation. Local field potentials (LFPs) usually developed abnormal spikes in the NAc and the anterior cingulate cortex (ACC). The behavioral manifestation could occur without obvious LFP spikes, however, when phase-phase coupling of alpha oscillations were accentuated between the NAc, ACC, and the primary motor cortex. These findings contrasted with myoclonic motor tics induced by disinhibition of the dorsolateral putamen, where PET activity was confined to the ipsilateral sensorimotor system and LFP spikes always preceded motor tics. We propose that vocal tics emerge as a consequence of dysrhythmic alpha coupling between critical nodes in the limbic and motor networks.
Suggested Reviewers:	James Leckman, MD, Phd Neison Harris Professor in the Child Study Center and Professor of Pediatrics an, Yale Medical School james.leckman@yale.edu Prof. James Leckman is a leading clinician and researcher who has focused on Tourette syndrome and has published numerous papers on the origin of premonitory urge. Kevin J Black, MD, Phd Washington University School of Medicine in St. Louis kevin@npg.wustl.edu Prof. Kevin J. Black. Is a leading clinical researcher in Tourette syndrome, and has

	<p>published numerous articles on the pathological basis of Tourette syndrome.</p> <p>Jonathan Mink, MD, PhD Frederick A. Horner, MD Endowed Professorship in Pediatric Neurology, University of Rochester Medical Center School of Medicine and Dentistry Jonathan_Mink@urmc.rochester.edu Prof. Jonathan Mink, is a world leading researcher in basal ganglia mediated movement disorders.</p>
<p>Opposed Reviewers:</p>	<p>Izhar Bar-Gad bargadi@mail.biu.ac.il Prof. Izhar Bar-Gad is a former work colleague and current direct competitor with respect to monkey models of Tourette syndrome.</p> <p>Leon Tremblay leon.tremblay@isc.cnrs.fr Prof. Leon Tremblay is a former work colleague and currently a direct competitor for monkey models of Tourette syndrome.</p>



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Oct 1, 2015

Dear Dr Furman,

Thank you for the chance to respond to the reviewer feedback for our recently submitted manuscript to Neuron. We have over the last 3 months endeavored to address all the comments made by our colleagues. Their suggestions, we feel, have greatly improved the original submission.

The advice of our colleagues to further investigate the properties of the local field potential (LFP) recordings during vocal tics has been particularly useful. One of the major additions to the manuscript includes the use of phase-phase coupling analysis to determine why vocal tics emerge in the absence of bicuculline driven LFP-spikes. We are pleased to announce that we can now identify a discrete alpha range (7-12 Hz) coupling between the limbic and motor regions that appears to drive the abnormal vocalizations recorded in our monkeys.

We hope that both yourself and our colleagues who reviewed the previous manuscript find the major revisions satisfactory and worthy of publication. I look forward to hearing from you.

Yours sincerely,

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McCairn et al., Reviewer Rebuttal

Reviewer Comments:

Reviewer #1: In this paper the authors test the hypothesis that vocal and motor tics are generated by distinct cortical-basal ganglia neural networks. They test the hypothesis by focal reversible inactivation of the nucleus accumbens (vocal tics) and sensorimotor putamen (motor tics) combined with PET imaging and multi-electrode neural recordings in non-human primate subjects. The problem they address is important because we do not have a good understanding of the pathophysiology of a disabling vocal tic disorder, Tourette's Syndrome, and no reliable animal model exists for this disease.

Based on the results of their experiments, the authors conclude that vocal and motor tics are generated by distinct cortical-basal ganglia pathways: vocal tics involve the nucleus accumbens, anterior cingulate and motor cortex, while motor tics are related to activity in sensorimotor putamen and motor cortex. They present three lines of evidence (reversible lesions, PET, and LFP) in support of their claims. The inactivation data using the GABA antagonist bicuculline are strong and show a clear association between the two different tics and their putative subcortical neural substrate. Similarly, the PET data taken after tics were induced by inactivation generally support the claims about the distinct circuits. The LFP data are a little more problematic; whereas the occurrence of LFP 'spikes' seem consistently related to the motor tics, there is no clear relationship between the spikes and the vocal tics. Did the authors interrogate the LFP data in more detail exploring whether there might be an association between well established LFP frequency bands and features of the behavioral data; if not this needs to be done.

Overall, I think that the authors provided strong support for the principal claims made in the paper and the work is a significant contribution to our understanding of the genesis of vocal tics. I suggest that the authors perform more detailed analysis of the LFP data and expand the discussion of this aspect of the results.

We would like to thank our colleague for their appraisal of our manuscript and their kind words with respect to the contribution the data could make to our understanding of vocal tics. With respect to their observations regarding vocal tics and LFP data, all members of our research group recognize that the weaker association between detectable LFP spikes and the emergence of vocalization is a problematic finding, especially when there is a clear association between LFP spikes and myoclonic tics. In order to address this issue, we have taken their suggestion to further interrogate the LFP data, with a specific focus on vocal tic phenomena, to determine if a coherent mechanism can be found which could explain the observed behavioral and electrophysiological phenomena.

As a consequence of this suggestion and new analysis, we feel that we are now able to identify and propose a fundamental mechanism for tic generation that is not dependent on the specific emergence of LFP spikes (p. 9 ln.191-218). Specifically, we have conducted a power spectral density analysis (PSD) and phase-phase coupling analysis on LFP data that is temporally associated with the emergence of vocal tics. This analysis identified a detectable increase in the

alpha (7 - 12 Hz) range in the PSD and phase-phase coupling that was clearly associated with the emergence of vocal tics (Figure 5A and Figure S4A and S4B). We believe this increase in the alpha range is a critical signature of tic generation in the monkey model, and is supported by recent observations taken in clinical studies, where direct electrophysiological recording of neural activity has also identified prominent alpha signaling (Marceglia et al. 2010; Zuber et al. 2014; Bour et al. 2014).

An initial analysis of periods where vocal tics emerged without detectable LFP spikes using PSD yielded no detectable change from control conditions. In order to investigate further the LFP signals, we used phase-phase coupling analysis. This particular analysis was chosen because it is able to identify significant spectral (phase) interactions independent of the actual power of the signal. As a consequence of this analysis, we can demonstrate that in the periods when vocalization occurs without detectable LFP spikes, there is an increase of phase-phase coupling in the alpha (7 - 12 Hz) range (Figure 5B and 5C). We believe that the identification of this signal provides a coherent mechanism independent of the LFP spikes which can drive the vocal tics observed in this experiment.

Again, we would like to thank our colleague for their suggestion to interrogate the LFP data further, as this has been instrumental in identifying this novel alpha signal and phase-phase coupling mechanism. We hope this novel analysis and new result answers their particular query regarding the nature of the LFP signaling in the bicuculline monkey model of TS.

Reviewer #2: These authors, a team that has published some of the most important animal model based neurophysiology of human motor tic disorders, provide captivating data that may reveal a dissociable mechanism for motor and vocal tics, the hallmark of the most clinically significant form of tic disorder, Tourette syndrome. Their impactful work using injection of a gaba antagonist into the dorsal putamen to replicate a putative model of tics, namely a decrease in gabaergic interneurons in the striatum, produced rather convincing orofacial tic-like behaviors in monkey.

The current manuscript under consideration reveals that a comparable paradigm including a "limbic" basal ganglia target, the NAc, produces behaviors that rather convincingly resemble human vocal tics.

These authors possess an array of methodological skills encompassing neurophysiology and PET allowing them a rather incisive examination of the differential effects on cortico-subcortical networks owing to manipulation of the dorsal putamen (motor tics) and the NAc (vocal tics). Most significant is the observation that the anterior cingulate and other brain regions implicated in limbic functioning are related to vocal tics, but not motor tics. This observation leads to the conclusion that the former are much more deeply related to emotion/motivation potentially explaining the greater clinical burden associated with vocal tics.

The data are novel, important, deeply interesting, and relevant to a general neuroscience audience.

However, numerous issues with the manuscript require significant attention. All of the critical comments that follow are readily addressable by the authors.

I will preface the listing of criticisms with a general point that influences the paper in its entirety. The authors describe the generated vocalization as akin to a simple vocal tic in humans. That interpretation seems incorrect. The vocal tics demonstrated---grunting--- appear to be a communicative vocalization described by those who study these macaques as indicating a submissive state on the part of the monkey making the sound. These grunts cannot be treated as akin to simple vocal tics in humans, such as sniffing and throat clearing. Vocal tics that have meaning, whether due to semantics in humans, or the social communicative calls of the macaque, should be considered complex. Accordingly, and importantly, the authors may have identified a mechanism for complex vocal tics, not simple vocal tics or vocal tics per se.

Page 2 line 30: Regarding this point, the authors make strong statements, including in the first sentence of the summary, pointing out that because vocalization is how we communicate socially, involuntary vocalization, i.e. vocal tics, leads to major clinical burden. But simple vocal tics like sniffing and throat clearing are not typically major causes of clinical burden. It is indeed the complex vocal tics, for the most part, that have that impact. By reframing the paper to be about complex vocal tics, the clinical burden argument will follow more logically.

We again would like to thank our colleague for their generous appraisal of this manuscript and kind words regarding our previous contributions to the field of tic disorders. We do recognize their concerns with respect to our categorization of the vocalization achieved in this study as a simple vocal tic. The subject of how to define the vocal phenomena seen in this study was the subject of intense debate between all members of our research group, and also the external experts that we consulted to try and identify the nature of the sounds induced by NAc bicuculline injection. The general consensus in our group was that, yes, the sounds are structured vocalizations that Macaques do use for communication, and as a research group which specializes in nonhuman primate studies, our initial assessment and feeling was to describe within the manuscript the vocalizations as complex vocal tics.

After several drafts of the manuscript were iterated and the discussion evolved, we felt as a group that as we were trying to make a comparison to human tic disorders and in particular Tourette syndrome, that if using face validity criteria, i.e., what exactly does the vocalization most closely resemble: it was concluded that a grunt would be, in the human context, most likely described as a simple tic. Therefore, we felt that describing the monkey vocal phenomena as a simple vocal tic would provide the least biased description of the vocalization, especially when comparing to equivalent human vocal phenomena, and our own biases towards work with primates. We are therefore encouraged that our colleague recognizes that the structured vocalization achieved by our model could be representative of a complex vocal tic. We have, in line with their suggestion, now modified the manuscript to reflect that the vocalization could be representative of a complex vocal tic

Page 2 line 36: The occasional emergence of the vocal tic without preceding lfp is an interesting

finding---discussed further below. One consideration is that it could be a simple vocal or motor tic phenocopy of the complex vocal tic. Patients with Tourette and verbal tics, particularly coprophenomena, will have quiet subvocalizations of louder more dramatic vocalizations. Conceivably, the vocal tic that lacks limbic LFP activity could be a version of such subvocalization. What happens in dorsal putamen during the vocal tics without preceding lfps?

Our colleague makes an interesting point about the possibility of the vocalization being a simple tic phenocopy of the complex tic. It is certainly true, that without some underlying mechanism being identified which could drive the behavior, which our colleague's phenocopy hypothesis could provide a valid theory as to why the phenomena emerges. We feel, however, that as we have now identified a clear phenomenon of alpha coupling/signaling associated with the vocalizations with or without LFP spikes (Figure 5) (p. 9 ln.191-218); that in this particular case, it is more appropriate to describe the general mechanism for vocal tic generation, rather than trying to describe more poorly understood clinical symptoms onto what is at this stage a very new finding with respect to tic encoding. In support of our reasoning we would like to point our colleague to (Figure 4C), we show that as a population the spectral properties of the vocalization are virtually identical in both frequency and power, whether vocal tics occur with or without LFP spikes. We would like to suggest that this identical spectral property indicates that the vocal tics are identical in nature and therefore represent the same phenomenon. This phenomenon, we believe is driven by the newly identified alpha phase-phase coupling. With respect to the putamen we have limited data from this region, but in the examples we have acquired there is minimal activity and limited phase-phase coupling between the NAc and putamen (Figure R1).

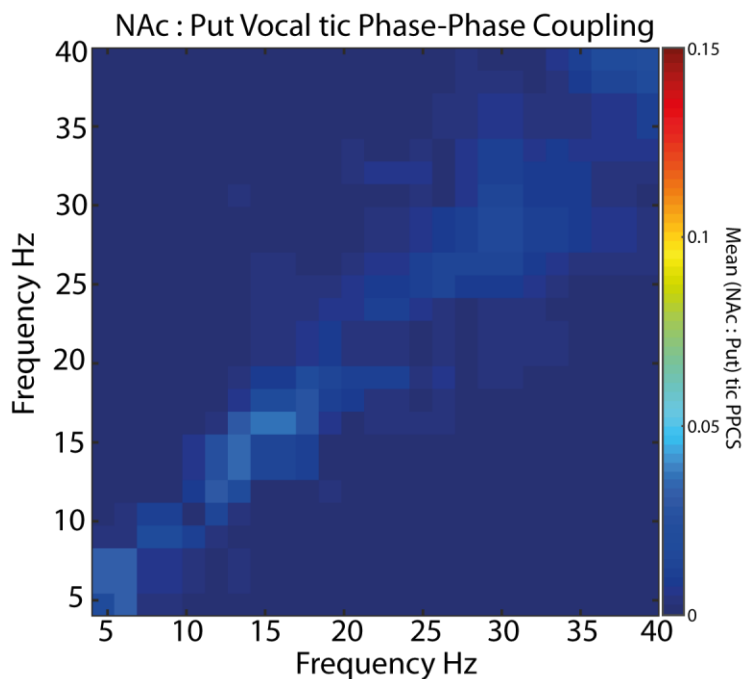


Figure R1. An example of phase-phase coupling analysis between the putamen and NAc demonstrating minimal interaction between the regions during the expression of vocal tics.

Page 3 line 51-56: As stated, it is important to distinguish tics with communicative intent/value from simple tics. Doing so will help clean up the confusion caused by making a strong argument about communication and then naming the vocal tics as "simple". The results will also be framed

more effectively. Also, it seems that the "devastating" impact is being pushed too hard. The vast majority of patients with vocal tics are not devastated by them.

We have amended the text of the document in line with our colleague's suggestion, the revised text can be found (p. 3 ln. 51-56).

The term vocalization denotes a range of vocal productions encompassing not only human speech and animal calls, but also nonverbal sounds – including laughing or crying, and emotional intonations related to fear, rage or threat. Other miscellaneous noises, such as throat clearing or coughing, under appropriate conditions, can be made elaborately to attract attention from, or convey communicative intentions to others. Given the importance of vocalizations, their dysfunction can lead to profound impacts on daily living.

Page 3 line 60-66: This long sentence should be re-casted. The last portion of the sentence seems to be making the point that vocal tics, as opposed to motor tics, are "more or less" semi-voluntary. But motor tics are semi-voluntary---that's why many patients can suppress them.

In line with this suggestion we have modified the text to just use the term 'tics', the revised text can be found (p. 3 ln. 64-66).

It is also controversial whether tics are generated in a purely involuntary fashion, or they are more or less "semi-voluntary" behavioral responses to uncontrollable impulses or urges (Kwak et al. 2003).

Page 4 lines 74 and 90: Understanding of the anterior cingulate cortex in monkey and human has increased substantially in recent decades. The ACC functions in emotional/motivational/limbic systems. It is also deeply important in cognitive and executive control. The ACC is not a single functional area. It comprises multiple functional areas that participate in multiple brain systems. Is the discussion related to the ventral ACC? The dorsal ACC? Clarity regarding these points, as well as showing anatomically precisely what part of the ACC the authors regard as limbic in the macaque is critical.

Our colleague raises an important point about the heterogeneity of the ACC with respect to the functional territories of this complex cortical structure, and the importance of recording from anatomically identified limbic regions. Our target for recording from limbic ACC was to record from area 24c in the macaque, an area that has been strongly implicated in limbic processing in the monkey (Morecraft and Van Hoesen 1998). Area 24c extends rostral and caudal to the corpus callosum and we endeavored to record from as much of the structure as was possible within the constraints of the internal space provided by the recording chamber. We have attached a figure to this document to illustrate some examples of our recording targets within the ACC (Figure R2). We have also clarified within the manuscript where precisely we recorded (p. 5 ln 103 p.7 ln

146, 160, p.8 ln 177, p9 ln 199, p18 in 389) and included sagittal sections from the PET imaging (Figure 3 and Figure S3) to better illustrate the regions we recorded from.

Gliosis from electrode guide penetration



Figure R2. Showing a demonstration of recording sites targeting the limbic region of the ACC – Area 24c. On the left a post-mortem histological section shows the gliosis left by the electrode piercing guide (rostral to the corpus callosum), faint vertical tracks left by the glass coated tungsten electrodes can be seen in area 24c. On the right a fusion MRI/CT X-Ray image of a recording electrode which has passed through ACC area 24c at the level of the corpus callosum.

Page 4 line 89: "sketchily" seems unnecessarily pejorative.

We agree and have modified the text accordingly; the revised text can be found (p. 4 ln. 87-89).

Firstly, it has been documented that injections of bicuculline into the associative or limbic striatum could induce vocalizations, albeit quite rarely (Worbe et al. 2009).

Page 5 line 118: Again, the vocal tic cannot be called simple if it has communicative intent.

Agreed, we have changed the text in line with the reviewer's suggestion; the revised text can be found (p. 5 – 6 ln. 117-122).

Our injection protocol for the NAc successfully evoked repetitive vocalizations (Figure 1A). The sound of their frequency spectrum was best described as a 'grunt' (Green 1975; Fukushima et al. 2014) (Figure 1C and Supplemental movie S1). As the vocalization

was structured and comparable to vocalization made by normal monkeys, we suggest the induced vocalizations are akin to a complex vocal tic in human patients (The Tourette Syndrome Classification Study Group 1993).

Page 7 line 158: Show the M1 trace for 4B.

We have modified the figure to include M1 activity, we have also included EMG activity to demonstrate that the vocalization is associated with clear orofacial muscle activity.

Page 7 line 161-162: Confusing. The prior sentence seems categorical---"the fact". This sentence, then, negates the categorical with 'however'. Are 4A and B from the same animal (i.e. "different occasions") or different animals?

We agree that the highlighted sentence could be confusing, we have modified the text so that the association between LFP spikes and vocalization seems less categorical (see below). The data shown is from the same animal, but on different occasions; the revised text can be found (p. 7 ln. 162-165).

This finding reflects the observation that not all LFP spikes triggered vocal tics, although, generally, each tic event was associated with an LFP spike in the ACC. Interestingly, however, on other occasions, vocal tics could readily occur without preceding LFP spikes (Figure 4B, gray rectangle).

Page 8 line 173 (and page 9 line 187): The interpretation that there is a "weaker causal relationship" seems somewhat insufficient. Given that, on occasion, the vocal activity precedes the NAc, could it be that direction of effect indicates that the NAc activity is in response to the vocalization?

The issue raised by our colleague that the LFP spike could be a response to vocalization is a valid point. We feel that the primary rebuttal to this point is that if vocalization was the causal factor in LFP spike generation, and the LFP spikes were in fact a consequence of movement artifacts within the recording systems, it would be not be possible to see vocal tics in which there is no LFP spike. We do recognize that the temporal jitter associated with LFP spikes and vocalization is a problem, however, now we have a mechanism by which vocal tics can emerge through alpha phase-phase coupling, we believe the temporal relationship between LFP spikes vocalization is much less of a confound. It could be conjectured that increased phase-phase coupling is the primary mechanism of vocalization and LFP spikes could be a consequence of increasing alpha phase-phase coupling, please (Figure 5) (p. 9 ln.191-218) for the updated results.

Page 9 line 191: Vocalization tic behavior with communicative value/intent is better described

as "complex".

Agreed, we have changed the text in line with the above suggestion; the revised text can be found (p. 10 ln. 220-222).

We have shown that disinhibition of a highly localized region of the NAc can consistently induce vocalizations in monkeys that bear a resemblance to complex vocal tics in TS patients.

Page 10 line 218-220: A more complete examination of M1 during the vocal tics that seem dissociated from limbic structure lfp activity could be very revealing.

We refer our colleague to the new spectral and phase-phase coupling analysis p.9 and (Figure 5). As a result we are now in a position to advance the concept that tic phenomena, especially those associated without LFP spikes can be driven by increased phase-phase coupling.

Page 10 line 222-223: Not all vocal tics are driven by heightened affective/motivational state. And certainly some motor tics are driven by such states. It is very important to avoid creating a false dichotomy. To date, the authors have a convincing model of simple motor tics and, with the present data it would seem, complex vocal tics. Simple vocal tics and complex motor tics have yet to be demonstrated.

In line with our colleague's suggestion and the updated results from the spectral and phase-phase coupling, we have substantially altered this segment of the text; the revised text can be found (p. 11 ln. 254-267).

In striking contrast with motor tics, the vocal tics that we observed may not be a direct behavioral consequence of LFP spikes. Rather, vocal tics maybe a consequence of the emergence of increased alpha signaling, this signaling occurs in LFP spike waveforms as indicated from the PSD, or can also occur as a response to elevated phase-phase coupling in the alpha frequency range. It is important to note this coupling can emerge without obvious voltage spikes from the background LFP activity. Increased coupling has been identified as a mechanism of information transfer between discrete networks (Belluscio et al. 2012; Fell and Axmacher 2011), and changes to discrete coupling frequencies has been observed in cortico-basal ganglia interactions in movement (de et al. 2013; Lalo et al. 2008; Dzirasa et al. 2010) and neuropsychiatric disorders (Bahramisharif et al. 2015). The identification of prominent changes to low-frequency oscillations in the alpha range has been observed in physiological recordings made from TS patients (Marceglia et al. 2010; Zauber et al. 2014; Bour et al. 2014), and so their identification in the bicuculline model of TS adds increasing support to the validity of this model as representative of the clinical condition.

Page 11 line 245-247: That false dichotomy is evident here as well. Both simple and complex motor tics have premonitory urges---such premonitory urges are not limited to vocal tics. The cited neuroimaging data, e.g. Neuner, does not support such a dissociation of motor and vocal tics.

We have removed the reference to only vocal tics, and the discussion about premonitory urges is now focused on tics in general; the revised text can be found (p. 13 ln. 290-295).

We propose that the activity associated with tics reported in this investigation, e.g., LFP spikes and the emergence of prominent alpha phase-phase coupling, is a neurophysiological correlate of the premonitory urge. Imaging studies in TS patients have identified paralimbic areas, e.g., ACC and amygdala, regions that were identified in our study, as being particularly active during premonitory urge and tic generation (Wang et al. 2011; Neuner et al. 2014; Bohlhalter et al. 2006).

Page 12 line 257-259: This conclusion, again, driven by a false dichotomy is not clinically valid and goes beyond the presently available data.

The text has been removed and the conclusion modified substantially due to the updated analysis regarding spectral and phase-phase coupling (p. 14 ln. 304-308).

We suggest that synchronized low-frequency dysrhythmia across key cortico-basal ganglia networks is a key feature of tic generation. Continuing efforts to determine precise mechanisms underlying vocal tic expressions will provide important insights into the structure and function of primate vocal control and open up a new avenue for translational research in TS.

Page 17 line 365: If there are more motor tics than vocal tics events, will that bias the PET estimated rCBF comparisons?

Our colleague raises an important point as to the nature of the estimated rCBF comparisons, i.e., if the rate of myoclonic tic expression is increased relative to vocal tics, would this bias the measurements. We feel that the measurements are comparable because the underlying neural dysrhythmia, i.e., the rate of LFP spikes is comparable in both conditions, and it is likely that the rCBF is most sensitive to the emergence of the LFP spikes, rather than emergent physical behavior.

Page 22 line 534-538: There is no explanation in the text or the legend of the method or purpose of "shuffling".

A description of why shuffling was undertaken has been added to the methods (p.20, line 437-440).

In order to visualize the mean CV the bins derived from the instantaneous CV were randomly shuffled to remove the large instantaneous variations in the CV. All the statistical procedures were carried out using Student's t-test. .

Page 23 line 542-547:

A) The supplemental figure 3s has different slices than figure 3. Figure 3 has 5 slices. Figure 3s has 6 slices. It seems that altogether there is a total of 7 slices represented by the two figures, but selected subsets are shown for each. A revised figure 3 and 3s should have all 7 slices. A full depiction of the anterior cingulate cortex from ventral anterior to dorsal, would be helpful as well, given the key point being made about a dissociation between vocal and motor. In addition, the figures should be clearly labeled showing 1) the locations of the structures examined as well as 2) the names of the locations of differential rCBF in each contrast. Alternatively, since motor > vocal does not mean "motor and not vocal", figure 3 could reasonably be expanded to include all of the contrasts in a single plate.

The figures have been modified in line with our colleague's suggestion, this includes sagittal sections so the full extent of activation in the ACC can be visualized.

B) Where exactly do the authors consider ACC? Are they referring to the entirety of the anterior cingulate or a particular portion of it?

Our recording was focused on the ACC area 24c, the recording region is summarized in the figure included with this rebuttal (Figure R2). We have also modified the main body of the text and methodology to describe where exactly in the ACC we were recording.

C) It is puzzling that the grunts do not produce rCBF changes in sensorimotor cortex, cerebellum and thalamus related to the sensory and motor aspects of the grunting behavior. What would the PET estimate of rCBF and the neurophysiology look like if one trained a macaque to grunt (e.g., voluntarily, to reward)?

The reviewer raises an important point relating to the absence of rCBF changes in sensorimotor cortex following NAc bicuculline infusion. The reason for this phenomena is that in order to confidently delineate the precise networks driven by the injection we utilize very high thresholding with the raw PET data. Please note that during limbic vocal tics there are corresponding dysrhythmias in the sensorimotor regions. If we use thresholding levels that allows identification of these elevated levels within sensorimotor cortex during limbic disruption, it becomes increasingly difficult to precisely visualize the key areas within the limbic regions due to the very strong response within the limbic networks. Bleed through of PET signals into adjacent structures and false positives become a problem when trying to interpret the data.

Page 23 line 551-556:

A) As noted, not all of the LFPs are linked to vocal tics. Is there absolutely no change in behavior associated with LFPs not linked to vocal tics? It does appear that the M1 trace has a low amplitude change temporally related to the LFPs recorded in NAc and ACC. This potential relationship should be examined.

We have examined the animal's behavior extensively during the periods questioned by our colleague, we are unable to detect any gross motor/behavioral changes associated with these LFP spikes, included below in the figure is a long data file which shows audio recording of vocal tics and EMG recording from the orofacial and arm region (Figure R3). It can be seen that although there is persistent vocalization and activation of facial muscles there is no detectable activity in the EMG taken from the bicep muscle. It is possible that there are subtle changes to the animals perceptual or cognitive processes, or if the animals was in a more naturalistic environment there might be some behavioral correlate which may be detectable. Our current experimental setup though, precludes us from conducting open field experiments where it is possible to acquire physiological signals and align them with behavioral effects induced by NAc injection.

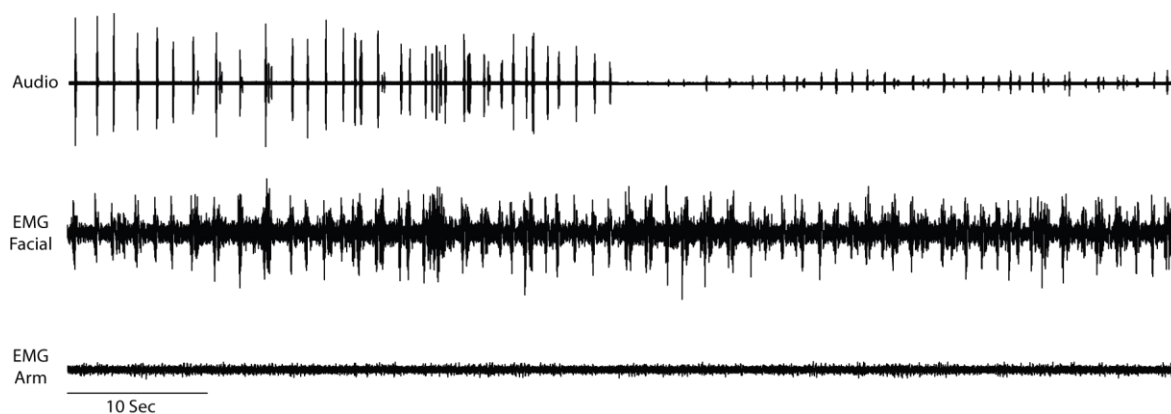


Figure R3. Showing a demonstration of vocal tics aligned to EMG activity from the orofacial and arm region. Note the large activations in the facial EMG and absence of activity in the EMG recorded from the arm.

B1) The gray bars do not capture all of vocalizations that are not linked to a strong LFP in ACC/NAc. These vocalizations are generally smaller amplitude than the grunts yoked to LFP changes. In addition, many of the grunts are "doublets" where the second grunt is typically smaller and not obviously linked to a clear LFP. The strong sense is that some of the vocalizations are not as yoked to the limbic system as others. These vocalizations are mostly of lower amplitude, consistent with the subvocalization notion discussed above.

B2) Where is the Motor cortex trace in B? It would be very interesting to see if a differential motor cortex LFP activity was found for those vocalizations that were not strongly yoked to the limbic regions. For example, some vocalizations may be communicative and emotive, while others might be "subvocalizations", physiologically and phenotypically closer to a simple motor tic.

We have substantially modified figure 4B to better clarify the phenomenon of vocalization occurring without LFP spikes. We have also included M1 and EMG recordings to better illustrate the phenomenon. We also refer our colleague to the new analysis with respect to the spectral and phase-phase coupling in lieu of an explanation for the subvocalization hypothesis raised by themselves.

C) There appear to be small LFP spikes in the ACC.

Our colleague is correct that there are indeed small LFP spikes in the ACC in response to myoclonic tics. This phenomenon mirrors exactly what occurs with NAc injections, i.e., small spikes appear in the motor cortices, time locked with the major spikes that occur in the limbic networks. This phenomenon is most likely a consequence of overlapping connections between the two functional regions. It is for this reason that we use very stringent threshold crossing criteria with the PET imaging data.

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1 **Title:** A primary role for nucleus accumbens and related limbic network
2 in vocal tics

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21 Running title: Multidimensional analyses of vocal tics

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29 **Summary:**

30 Inappropriate vocal expressions – e.g., vocal tics in Tourette syndrome – severely impact quality
31 of life. Neural mechanisms underlying vocal tics remain unexplored because of no established
32 animal model representing the condition. We report unilateral disinhibition of the nucleus
33 accumbens (NAc) generates vocal tics in monkeys. Whole-brain PET imaging identified
34 prominent, bilateral limbic cortico-subcortical activation. Local field potentials (LFPs) usually
35 developed abnormal spikes in the NAc and the anterior cingulate cortex (ACC). The behavioral
36 manifestation could occur without obvious LFP spikes, however, when phase-phase coupling of
37 alpha oscillations were accentuated between the NAc, ACC, and the primary motor cortex. These
38 findings contrasted with myoclonic motor tics induced by disinhibition of the dorsolateral
39 putamen, where PET activity was confined to the ipsilateral sensorimotor system and LFP spikes
40 always preceded motor tics. We propose that vocal tics emerge as a consequence of dysrhythmic
41 alpha coupling between critical nodes in the limbic and motor networks.

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43 (150 words)

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50 **Introduction:**

51 The term vocalization denotes a range of vocal productions encompassing not only
52 human speech and animal calls, but also nonverbal sounds – including laughing or crying, and
53 emotional intonations related to fear, rage or threat. Other miscellaneous noises, such as throat
54 clearing or coughing, under appropriate conditions, can be made elaborately to attract attention
55 from, or convey communicative intentions to others. Given the importance of vocalizations, their
56 dysfunction can lead to profound impacts on daily living. In Tourette syndrome (TS), as well as
57 simple motor tics, patients often suffer from irrepressible attacks of vocalizations. Vocal tics
58 range from simple forms, e.g., throat clearing, grunting, etc., to complex – such as swearing
59 (coprolalia), or other socially inappropriate outbursts (Robertson et al. 2009; The Tourette
60 Syndrome Classification Study Group 1993). Unlike motor tics generated by the “sensorimotor
61 loop” of the cortico-basal ganglia network (Figure S1A) which have been extensively
62 investigated in the monkey (Bronfeld et al. 2011; McCairn et al. 2009; McCairn et al. 2013b), it
63 remains unclear what neural networks and mechanisms are responsible for the expression of
64 vocal tics. It is also controversial whether tics are generated in a purely involuntary fashion, or
65 they are more or less “semi-voluntary” behavioral responses to uncontrollable impulses or urges
66 (Kwak et al. 2003).

67 In nonhuman primates, vocalization is under the control of two hierarchically organized
68 neural pathways (Jurgens 2009). One pathway runs from the anterior cingulate cortex (ACC) via
69 the periaqueductal gray into the reticular formation, which in turn innervates phonatory
70 motoneurons in the brainstem and spinal cord. The second pathway runs from the primary motor
71 cortex (M1) via the reticular formation to phonatory motoneurons. Consistent with these
72 anatomical investigations, the ACC and M1, in monkeys, display readiness potentials preceding

73 voluntary utterance (Gemba et al. 1995). This argument points to the importance of the ACC and
74 M1 in vocal control. The M1 constitutes part of the sensorimotor cortico-basal ganglia loop
75 (Alexander et al. 1986) that is responsible for the expression of motor tics (Bronfeld et al. 2011;
76 McCairn et al. 2009; McCairn et al. 2013b), whereas the ACC participates in a different cortico-
77 basal ganglia circuit, called the “limbic loop” that is involved in emotional and motivational
78 processing (Figure S1A) (Alexander et al. 1986; Morecraft and Van Hoesen 1998). One may
79 therefore view that responsible networks and mechanisms for vocal tics do not fundamentally
80 differ from those for motor tics. According to this view, the only difference resides in the
81 affected body part. However, this view is not immediately supported by the existing literature
82 (Bronfeld et al. 2011; McCairn et al. 2009; McCairn et al. 2013b; Worbe et al. 2009). The
83 behavioral phenotype in the primate TS model has so far been confined to motor tics. No studies
84 have consistently evoked vocal tics using animal models affecting the sensorimotor loop.

85 We therefore tested another hypothesis, that vocal tics might in fact be produced by
86 abnormalities in the limbic loop that involves the ACC. Our hypothesis is supported by three
87 independent behavioral studies in monkeys. First, it has been documented that injections of
88 bicuculline into the associative or limbic striatum could induce vocalizations, albeit quite rarely
89 (Worbe et al. 2009). Second, electrical stimulation in the ACC, a critical node in the cortical
90 limbic network, induces vocalizations (Jurgens and Ploog 1970; Robinson 1967; Smith 1945).
91 Third, bicuculline injections into the limbic thalamus can elicit abnormal vocalizations (Rotge et
92 al. 2012). Furthermore, in patients with TS who manifested both vocal and motor tics, positron
93 emission tomography (PET) imaging has identified activation of the ACC, along with other
94 cortical motor areas (Stern et al. 2000). These observations raise the possibility that vocal tics
95 should be considered a disorder of the limbic territory, as opposed to the sensorimotor territory,

96 of the primate cortico-basal ganglia networks. In order to test this hypothesis, the present study
97 carried out multidimensional analyses integrating behavioral characterization, neuroimaging, and
98 electrophysiological recording. We first show that the injection of bicuculline into the nucleus
99 accumbens (NAc), a central component of the limbic striatum (Haber and Knutson 2010), was
100 indeed capable of inducing vocal tics in monkeys. Whole-brain PET imaging revealed prominent
101 activation in the ACC, amygdala, and hippocampus, confirming the involvement of the limbic
102 network. Electrophysiological recording showed repetitive neuronal discharges in the NAc and
103 ACC (area-24c) associated with vocal tic generation. Furthermore, recorded local field potential
104 (LFP) showed an increased phase-phase coupling of alpha oscillations between the NAc, ACC
105 and M1 during vocal tic behavior. These multidimensional investigations now enabled us to
106 identify the similarities and differences between our vocal tic and motor tic model.

107

108 **Results:**

109 In order to disrupt physiological activity in the limbic and sensorimotor networks, we
110 injected a small amount of the GABA antagonist bicuculline into the NAc (limbic) or the
111 putamen (sensorimotor) (Figures S1B and S1C) in five monkeys (see [Experimental procedures](#)).
112 This pharmacological protocol was chosen among others (Bronfeld et al. 2013; Godar et al.
113 2014; McCairn et al. 2013a; McCairn and Isoda 2013; Worbe et al. 2013), because (i) tic
114 disorders in TS are hypothesized to arise from dysfunctional, local GABAergic circuits (Draper
115 et al. 2014; Kalanithi et al. 2005; Kataoka et al. 2010; Lerner et al. 2012) and (ii) the effect of
116 bicuculline is rapid, thereby bypassing concerns associated with compensatory mechanisms
117 (McCairn et al. 2013b; Worbe et al. 2009). Our injection protocol for the NAc successfully
118 evoked repetitive complex vocalizations (Figure 1A). The sound of their frequency spectrum was

119 best described as a ‘grunt’ (Fukushima et al. 2014; Green 1975) (Figure 1C and Supplemental
120 movie S1). As the vocalization was structured and comparable to vocalization made by normal
121 monkeys, we suggest that the induced vocalizations are akin to a complex vocal tic in human
122 patients (The Tourette Syndrome Classification Study Group 1993). The site that caused vocal
123 tics was consistently localized in the NAc across all the monkeys, i.e., approximately 4 mm
124 rostral to the anterior commissure (Figure 1D, left). To elicit motor tics, the bicuculline injections
125 had to be placed in the dorsolateral sensorimotor putamen (Figure 1D, right), caudal to the
126 anterior commissure. In such cases (n = 4 monkeys) where repetitive tics occurred in the
127 orofacial region (Figure 1B and Supplemental movie S2) and/or the arm region (Figure S1D and
128 Supplemental movie S3), no vocal tics were ever observed. The average duration of individual
129 motor tics was 780 ms, which was significantly longer than that of vocal tics (254 ms; $p <$
130 0.0001, t-test; Figure S1E). The localization of vocal tics to the NAc supports the premise that
131 vocal tics emerge as a consequence of limbic network dysrhythmia.

132 To compare the behavioral properties between vocal and motor tics, we plotted time-
133 dependent changes of inter-tic intervals in a representative session for vocal tics (Figure 2A) and
134 motor tics (Figure 2B). In the exemplified cases, vocal tics tended to emerge every 2 to 4 s, most
135 typically seen in the period between 400 s and 1200 s following the drug delivery (Figure 2A).
136 By contrast, motor tics tended to emerge every 1 s or so (Figure 2B). Our quantitative analysis
137 across the sessions showed that, on average, the inter-tic interval was significantly longer during
138 vocal tics [3.4 ± 3.3 s (mean \pm SD)] than during motor tics (1.8 ± 1.3 s; $p < 0.0001$, t-test; Figure
139 S2A). The occurrence of tics shifted back and forth between more regular states (lower
140 coefficient of variance (CV)) and more random states (higher CV) during both vocal tics (black
141 broken line) and motor tics (red broken line) (Figure 2C). However, the average CV was

142 significantly higher in vocal tics [black solid line; 0.69 ± 0.33 (mean \pm SD)] than in motor tics
143 (red solid line; 0.63 ± 0.21 ; $p < 0.0001$, t-test), although the difference was numerically small.

144 Our next step was to identify more globally which brain regions were activated
145 following disinhibition of the NAc. We found that regional cerebral blood flow (rCBF)
146 significantly increased in the ACC, especially (area-24c), amygdala, and hippocampus,
147 bilaterally (T value > 3.37 , uncorrected $p < 0.001$; [Figure S3](#)). This activation pattern was unique
148 to the vocal tic model; in the motor tic model, significant increases in rCBF were observed in the
149 M1 on the side ipsilateral to the injection site and in the cerebellum on the contralateral side
150 ([Figure S3](#)). The contrasting activation profile was best captured by a direct comparison of rCBF
151 between the two tic models. The ACC, amygdala, and hippocampus were each activated
152 significantly more strongly in the vocal tic model than in the motor tic model (T value > 5.47 ,
153 corrected $p < 0.05$) ([Figure. 3](#)). By contrast, M1 and the cerebellum were activated significantly
154 more strongly in the motor tic model (T value > 5.47 , corrected $p < 0.05$).

155 What might be the physiological basis for the over activation of rCBF in the limbic
156 network? To answer this question, we performed multisite recordings of local field potentials
157 (LFPs). We found that immediately after the bicuculline delivery into the NAc, repetitive large
158 deflections of LFPs — an electrophysiological marker of aberrant neuronal discharges called
159 LFP spikes (McCairn et al. 2009; McCairn et al. 2013b) — emerged in both the injection site and
160 the ACC (area-24c) ([Figure 4A](#)). The LFP spikes were also identifiable in the M1, but their
161 amplitude was much smaller ([Figure 4A](#)). Importantly, the occurrence of LFP spikes in the ACC
162 (and also the NAc) outnumbered that of vocal tics. This finding reflects the fact that not all LFP
163 spikes triggered vocal tics, although each tic event was preceded by an LFP spike in the ACC.
164 Interestingly, however, on other occasions, vocal tics could readily occur without preceding LFP

165 spikes (Figure 4B, gray rectangles). It should also be noted here that the spectrographic feature
166 of vocal tics was qualitatively similar irrespective of the existence of preceding LFPs (Figure 4C).

167 In the motor tic model, following bicuculline injections into the dorsolateral
168 (sensorimotor) putamen, prominent LFP spikes were identified in the M1 as well as in the
169 injection site (Figure 4D). Crucially, the number of LFP spikes in the M1 (and also the putamen)
170 was comparable with that of motor tics (Figure 4D), indicating that the occurrence of LFP spikes
171 corresponds well to emergence of behavioral tics. When we performed spike-triggered averaging
172 of EMG records using LFP spikes in the dorsolateral putamen (Figure 4E), there was a clear,
173 single peak of tic-related EMG that immediately *followed* the LFP spike onset (time = 0).
174 However, when the same analysis was performed for vocal records using LFP spikes in the NAc
175 as a trigger, there were two peaks in the vocal activity, with the first peak *preceding* the LFP
176 spike onset (Figure 4F), indicative of a weaker causal relationship.

177 The size of LFP spikes was significantly smaller in the ACC (area-24c) during vocal tics
178 than in the M1 during motor tics ($p < 0.0001$, t-test; Figure S2B). Shorter LFP spikes in the ACC
179 mirror the shorter length of vocal tics (Figure S1E). The size of LFP spikes in the NAc vs. the
180 putamen was comparable in the two tic states (Figure S2B). The temporal interval between
181 individual LFP spikes, i.e., inter-LFP-spike intervals, was significantly longer in vocal tics than
182 in motor tics ($p < 0.0001$, t-test; Figure S2C). This observation also reflects the longer inter-tic
183 interval in the vocal tic model (Figure S2A). Like behavioral tic expressions, the occurrence of
184 LFP spikes shifted back and forth between more regular states and more random states. However,
185 such a transition was less pronounced in vocal tics (Figure 2D) than in motor tics (Figure 2E).
186 Indeed, the regularity of LFP spikes was, on average, significantly higher in the vocal tic state
187 [CV, 0.32 ± 0.03 (mean \pm SD)] than in the motor tic state (CV, 0.47 ± 0.04 ; $p < 0.0001$, t-test;

188 [Figure 2F](#)). The finding that vocal tics occurred more irregularly ([Figure 2C](#)) despite more
189 regular expressions of LFP spikes ([Figure 2F](#)) may represent a seemingly weaker causal coupling
190 between neural and behavioral events for vocal tic generation, as described above.

191 Therefore, there are two electrophysiological conditions in which vocal tics occur, i.e.,
192 with and without LFP spikes. To better understand what is happening in the cortico-basal ganglia
193 networks during vocal tics, we carried out more detailed analyses using LFP data, specifically
194 power spectral density (PSD) and phase-phase coupling. A specific goal of these analyses was to
195 try and elucidate why animals could generate vocal tics in the absence of LFP spikes. In order to
196 address this, we first attempted to find out neural signatures, other than LFP spikes, that were
197 associated with the genesis of vocal tics when clear LFP spikes were observed. Initially, LFP
198 spikes that were definitively associated with vocal tics were extracted from the data set, and
199 PSD's were calculated on the LFP data from the NAc, ACC (area-24c) and M1, and compared to
200 LFP's acquired in the pre-injection stage. The analysis revealed two significant findings: for each
201 of the investigated regions there was an increase in the power of the PSD in the alpha range (7 -
202 12 Hz), which was particularly prominent in the NAc and ACC ([Figure 5A](#)). As a corollary of
203 this signal, we also identified increased phase-phase coupling in the same range between the
204 NAc and the limbic/motor cortices ([Figure S4A and Figure S4B](#)). We performed the same
205 analyses on the LFP data associated with vocal tics where no LFP spikes were evident, and
206 found no detectable increase relative to control data in the PSD analysis of the NAc, ACC and
207 M1 (data not shown). When using phase-phase coupling, however, it can be seen in the phase-
208 phase coupling plots that (NAc : ACC) and (NAc : M1) both show elevated coupling in the alpha
209 frequency band ([Figure 5B and Figure 5C](#)). A statistical analysis of the alpha frequency band
210 showed that the observed elevation in alpha was significant for (NAc : ACC) [tic PPCS, $0.26 \pm$

211 0.006 vs. control PPCS, 0.23 ± 0.007 ; $p = 0.0034$, t-test; (mean \pm SEM)] (Figure 5D). A similar
212 result was observed for the (NAc : M1) pairing [tic PPCS, 0.28 ± 0.008 vs. control PPCS, $0.24 \pm$
213 0.007 ; $p < 0.0001$, t-test; (mean \pm SEM)] (Figure 5D). The low beta range (13 - 20 Hz) was not
214 significantly different relative to control data for all pairings; however, in the high beta range (21
215 - 40 Hz) there was a significant drop in beta phase-phase coupling between the (NAc : M1)
216 pairing [tic PPCS, 0.22 ± 0.007 vs. control PPCS, 0.30 ± 0.005 ; $p < 0.0001$, t-test; (mean \pm
217 SEM)] (Figure 5D). We conjecture that increased alpha phase-phase coupling is a primary
218 mechanism for, and the most sensitive measure of, the genesis of vocal tics.

219 **Discussion:**

220 We have shown that disinhibition of a highly localized region of the NAc can
221 consistently induce vocalizations in monkeys that bear a resemblance to complex vocal tics in TS
222 patients. In our model, the expression of vocal tics was acute and reversible, as was the case with
223 motor tics caused by disinhibition of the dorsolateral putamen. The temporal dynamics of drug
224 effects enabled us to minimize concerns associated with compensatory mechanisms. The whole-
225 brain PET imaging demonstrated that effects of local pharmacological manipulation extended
226 broadly to affect several cortico-subcortical regions in the limbic network, bilaterally within the
227 cortico-basal ganglia networks. The network abnormality in the vocal tic model was in a marked
228 contrast to that seen in the motor tic model, where the most conspicuous activation was confined
229 to the sensorimotor network, ipsilateral within the cortico-basal ganglia network. Based on the
230 observed increases in rCBF, especially in basal ganglia-recipient regions of the cortex, multisite
231 recordings of LFPs identified repetitive LFP spikes although there were notable differences in
232 the underlying properties of LFP spikes associated with each tic type. We also identified a
233 discrete abnormality within the alpha frequency band (7 - 12 Hz) that was associated with vocal

234 tic generation, and was present as increased phase-phase coupling between the NAc, ACC and
235 M1 when the animal expressed vocal tics without obvious LFP spikes. The present study is the
236 first demonstration of the temporal and spatial structure of primate vocal tics and the direct
237 comparison of neural network dynamics between the vocal and the motor tic state under the
238 same experimental condition.

239 It is noteworthy that the generation of LFP spikes and the expression of behavioral tics
240 were more closely associated in motor tics than in vocal tics. A less direct causal relation for
241 vocal tics was indicated by two important observations. First, during the most intense phases of
242 the experiment, the occurrence of LFP spikes was not always followed by vocalization, unlike as
243 occurs with motor tics. A plausible explanation for this observation is that the M1 has direct
244 corticobulbar and corticospinal connections that innervate motor neurons for the control of fast
245 movement (Dum and Strick 1991; Shinoda et al. 1981), whereas the ACC controls vocalization
246 via multisynaptic pathways (Jurgens 2009). Thus, aberrant neuronal discharges in the M1,
247 associated with motor tics, would be more readily capable of triggering tic expressions than in
248 the ACC. In addition, the amplitude of LFP spikes was significantly larger in the M1 than in the
249 ACC (see [Figure S2B](#)), and this may also contribute to more efficient production of motor tics
250 following LFP spikes.

251 Second, vocal tics can occur without associated LFP spikes in the NAc, ACC – (area
252 24c), and M1. This observation seems puzzling as such, but may provide an important clue to
253 clarifying the fundamental nature of vocal tics, and the neural mechanism driving the behavior.
254 In striking contrast with motor tics, the vocal tics that we observed may not be a direct
255 behavioral consequence of LFP spikes. Rather, vocal tics maybe a consequence of the emergence
256 of increased alpha signaling. This signaling occurs in LFP spike waveforms as indicated from the

257 PSD, or can also occur as a response to elevated phase-phase coupling in the alpha-frequency
258 range. It is critical to note that such coupling can emerge without obvious voltage spikes from
259 the background LFP activity. Increased coupling has been identified as a mechanism of
260 information transfer between discrete networks (Belluscio et al. 2012; Fell and Axmacher 2011),
261 and changes to discrete coupling frequencies has been observed in cortico-basal ganglia
262 interactions in movement (de Hemptinne et al. 2013; Dzirasa et al. 2010; Lalo et al. 2008) and
263 neuropsychiatric disorders (Bahramisharif et al. 2015). The identification of prominent changes
264 to low-frequency oscillations in the alpha range has been done in physiological recordings from
265 TS patients (Bour et al. 2015; Marceglia et al. 2010; Zauber et al. 2014), and so their
266 identification in the bicuculline model of TS adds increasing support to the validity of this model
267 as representative of the clinical condition.

268 This increased alpha signaling may reflect changes in the internal emotional state of the
269 animal. A likely hypothesis concerning the mechanism of internal state changes is that the
270 infusion of bicuculline into the NAc may lead to an increase in the basal level of dopamine
271 release in the limbic network via activation of NAc neurons (Haber 2003; Haber and Knutson
272 2010). In favor of this hypothesis, focal application of bicuculline in the NAc of the rat elicited a
273 significant increase in extracellular dopamine in the NAc in a dose-dependent manner (Yan
274 1999). Such increases of extracellular dopamine may cause a change in alertness or motivation
275 (Bromberg-Martin et al. 2010), which could in turn trigger/facilitate vocalization. This
276 hypothesis also fits well with a previous report in monkeys showing that readiness potentials in
277 the ACC preceding voluntary utterance become significantly larger as the animals' motivation to
278 vocalize increases (Gemba et al. 1995). The preferential activation of the amygdalo-hippocampal

279 complex, as observed in their study, may also increase emotional/motivational saliency that is
280 intimately associated with a subset of vocalization behaviors.

281 Tics as a reaction to heightened emotional/motivational states may be described as a
282 “semi-voluntary” expression of abnormal behavior. Clinically, tics are often defined as being
283 semi-voluntary, as opposed to involuntary (Kwak et al. 2003; The Tourette Syndrome
284 Classification Study Group 1993), because their expression is, in some degree, under volitional
285 control (Cohen and Leckman 1992). Tics can sometimes be consciously suppressed, and are
286 frequently experienced as an irresistible urge that must be expressed, at some point, to relieve
287 any underlying psychic tension (Dure and DeWolfe 2006; Leckman et al. 1993). The conscious
288 experience of being aware of the urge to tic is commonly referred to as a premonitory urge. Such
289 semi-voluntary aspects of tics have been generally observed in TS patients, although their
290 underlying neural mechanisms remain largely unknown. We propose that the activity associated
291 with tics reported in this investigation, e.g., LFP spikes and the emergence of prominent alpha
292 phase-phase coupling, is a neurophysiological correlate of the premonitory urge. Imaging studies
293 in TS patients have identified paralimbic areas, e.g., the ACC and the amygdala, regions that
294 were identified in our study, as being particularly active during premonitory urge and tic
295 generation (Bohlhalter et al. 2006; Neuner et al. 2014; Wang et al. 2011).

296 In conclusion, TS is a multifaceted disorder that shows a wide range of symptom
297 profiles. The clarification of pathophysiology underlying each of the symptom profiles and their
298 integration will promote a better understanding of TS and improve the quality of life for patients
299 with TS. The present study demonstrates that bicuculline-mediated disinhibition of the NAc in
300 monkeys can cause vocal tics, one of the most troubling symptoms in TS. Although neural
301 underpinnings of vocal tics may share similar properties with those of motor tics, i.e., repetitive

302 LFP spikes in the cortico-basal ganglia networks, the causal relationship between LFP spikes and
303 tic occurrence is seemingly more complicated in vocal tics, with elevated alpha phase-phase
304 coupling appearing to drive expressed tics when no LFP spikes are evident. We suggest that
305 synchronized low-frequency dysrhythmia across the cortico-basal ganglia networks is a key
306 feature of tic generation. Continuing efforts to determine precise mechanisms underlying vocal
307 tic expressions will provide important insights into the structure and function of primate vocal
308 control and open up a new avenue for translational research in TS.

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322 ***Experimental Procedures***

323 *Animals*

324 Three male Japanese macaques (*Macaca fuscata*, designated R, B and C) and two male
325 rhesus macaque (*Macaca mulatta*, designated A and S) were used in this study. The animals'
326 health was monitored by a veterinarian, and fluid consumption, diet, and weight were monitored
327 daily. All procedures for animal care and experimentation were approved by the Institutional
328 Animal Care and Use Committee of Primate Research Institute, Kyoto University (Permission
329 Number: 2010-080), National Institute of Radiological Science (Permission Number: 09-1035),
330 the University of Tsukuba Animal Experiment Committee (Permission Number: 13-249) and
331 RIKEN Brain Science Institute (Permission Number: H22-2-216), and were in accordance with
332 the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

333 *Surgical procedures*

334 The surgical procedures for cranial implantation were conducted under aseptic conditions
335 using isoflurane (1.0-3.0 %) or pentobarbital (30 mg/kg IV) anesthesia after induction with
336 ketamine HCl (10 mg/kg IM). A square polyetherimide chamber (27 mm x 27 mm) was used for
337 targeting the M1 and basal ganglia structures. In two monkeys (R and B), the chamber was tilted
338 at 30° in the coronal plane, with the center targeted to the middle of the globus pallidus:
339 stereotactic coordinates at A21, L7 and H15 (Kusama and Mabuchi 1970). In the other monkeys
340 (A, C and S), we placed the chamber using a dorsoventral approach to allow for access to the
341 ACC region. The chamber was implanted stereotaxically on the animals' left side and fixed into
342 place using plastic bone screws and methyl-methacrylate cement. In addition, a head-holder

343 connector and an electroencephalogram screw were attached to the skull. Prophylactic antibiotics
344 and analgesics were administered postsurgically.

345 Following surgery, seven Tesla T2-weighted magnetic resonance images (MRIs) were
346 acquired (Magnet: Kobelco and JASTEC, Japan; Console: Bruker Biospin, Germany) and
347 combined with data from X-ray computed tomography (CT; Accuitomo170, J.MORITA Co.,
348 Japan) imaging to allow for *in vivo* determination of injection locations, recording sites, and PET
349 signals.

350 *PET procedures*

351 PET scans were acquired from monkey S under conscious conditions. All PET scans were
352 performed using an SHR-7700 PET scanner (Hamamatsu Photonics K.K.Inc., Japan) in two-
353 dimensional mode. Transmission scan with a rotating ^{68}Ge - ^{68}Ga pin source was collected for 50
354 min at the beginning of each day for attenuation correction. The emission scan was started when
355 a rising brain radioactivity count was first detected (>30 kcps) after the bolus intravenous
356 injection of ^{15}O -labelled water (about 1.11 GBq) via the crural vein using an automated water
357 generator (A&RMC, Melbourne, Australia). Each emission scan was performed for 120 s with
358 12 time frames at 10 s.

359 On injection days a Microdrive (MO-97A; Narishige, Japan) was used to place the
360 injection cannula while injections were targeted to the sensorimotor putamen ($n = 2$) or NAc ($n =$
361 6). Prior to injections, a control scan was captured; bicuculline was then infused at a rate of 2
362 $\mu\text{l}/\text{min}$ with a maximal volume of 7 μl delivered depending on the intensity of the effect. Total 8-
363 12 individual scans were acquired in each day.

364 We obtained a total of 69 scans. For the control condition, we used 9 scans from 8 days. For
365 the injection condition, we used scans obtained on days in which tics were evoked (putamen, 14
366 scans from 2 days; NAc, 12 scans from 2 days). All emission data were summated for the first 60
367 s and were reconstructed using filtered back projection with a 4.0-mm Hanning filter.

368 *Behavioral procedures*

369 During electrophysiological recording described below, the animals sitting in a primate chair
370 were allowed to complete a simple, visually guided reach task for a liquid reward. This task was
371 introduced merely in order to have the monkeys calmly sit in the chair during 2-3 hours of
372 neuronal recordings. Spontaneous behaviors and task execution were monitored and recorded
373 using a multichannel video system (GV-800, Geovision, Taiwan). The system was designed to
374 visualize behavioral sequences from four different locations, each capturing the face, upper limbs,
375 lower limbs, and the behavioral task. Digital images from each of the separate cameras were
376 captured at 25 frames per second and stored on a hard disk for offline analysis. Audio signals
377 were recorded with a standard audio microphone (Sony, Tokyo, Japan) at a sample rate of 8 KHz
378 combined with Tucker Davis RZ5 data acquisition module (Tucker Davis Technologies, Alachua,
379 FL, USA). In addition, electromyogram (EMG) signals were sampled from four muscles: biceps
380 brachii, triceps brachii, zygomaticus major, and ventral orbicularis oris. EMG wires were
381 constructed from 100- μ m Teflon-coated silver wire (A-M systems, WA, USA). The EMG wires
382 were percutaneous and inserted just prior to each experimental session using a 27-gauge
383 hypodermic needle. EMG signals were sampled at 5 kHz and band-pass-filtered (5-450 Hz, 4
384 pole Butterworth filter).

385

386 *Electrophysiological procedures*

387 Following recovery from surgery, the animals underwent combined MRI/CT imaging to
388 determine the position of the chamber relative to underlying brain structures. Then
389 microelectrode-guided mapping of structures of interest, e.g., NAc, M1, ACC (area-24c) was
390 undertaken. Each region of interest was identified by their characteristic neuronal activity and
391 their relation to known anatomical boundaries, as described previously (McCairn et al. 2013b;
392 McCairn and Turner 2009), and compared with a standard stereotaxic atlas (Kusama and
393 Mabuchi 1970). The preliminary mapping process was followed by experimental sessions.
394 During each experimental session, up to nine glass-coated tungsten microelectrodes (250-750 k Ω
395 at 1 kHz) and an injectrode [28-gauge cannula surrounding a Parylene-coated 50 μ m
396 microelectrode extending 0.5 mm beyond the tip of the injection cannula (Alpha Omega
397 Engineering, Nazareth, Israel)] were introduced. The electrode-manipulating system could move
398 each electrode independently with 2- μ m resolution (DMT, Flex-MT and EPS, Alpha-Omega
399 Engineering). The microinjection cannula was connected via a Delrin manifold to a 25- μ l
400 syringe (Hamilton Company, Reno, NV, USA) filled with bicuculline (Sigma-Aldrich, Japan).
401 The depth of the injection site within the striatum was determined by electrophysiological
402 recording using a microelectrode within the injectrode and confirmed by combined MRI/CT
403 images. All electrophysiological data were passed through a low-gain 16-channel headstage (2
404 Hz to 7.5 kHz band pass) and then digitized at 24 kHz (16-bit resolution; Tucker Davis
405 Technologies, Alachua, FL, USA).

406 LFP data were extracted by band pass filtering (8 pole Butterworth filter, cutoff at 2 - 300 Hz,
407 sample rate 5 KHz) of the wide-band extracellular signal. Once the recording electrodes and
408 injectrode were in place, bicuculline dissolved in physiological saline (15 μ g/ μ l) was injected (2

409 $\mu\text{l}/\text{min}$) initially up to 4 μl into the dorsolateral putamen or the NAc. If the resulting behavioral
410 effect was absent or weak, or prolongation of the behavioral effect was necessary for recording
411 purposes, additional perfusions of bicuculline (1-2 μl each time) were performed until the
412 maximum volume of 8 $\mu\text{l}/\text{day}$.

413 *PET data analyses*

414 Statistical analyses were performed with SPM8 software (Wellcome Department of
415 Cognitive Neurology, London, UK; www.fil.ion.ucl.ac.uk) and MATLAB R2013b (MathWorks
416 Inc., Natick, MA, USA). The reconstructed images were realigned and resliced for motion
417 correction. The resultant images were smoothed with a 3.0-mm Gaussian filter and then were
418 scalped. The global activity for each scan was corrected using grand mean scaling, using an
419 analysis of covariance for global normalization. The difference in the relative regional cerebral
420 blood flow between control and two injection conditions was statistically tested in each voxel.
421 Statistical threshold was set at $p < 0.001$, uncorrected ($T > 3.37$) or $p < 0.05$, corrected ($T > 5.47$).
422 To determine the anatomical localization of activated regions, we co-registered the PET data
423 with the individual MRI.

424 *Electrophysiological data analyses*

425 LFP data were processed offline and correlated with behavioral events using MATLAB and
426 Neuroexplorer (V4, Nex technologies, Littleton, MA, USA). The LFP signal was rectified and
427 down sampled by a factor of ten. A Hilbert transform was conducted to extract the envelope of
428 the acquired signal; the signal then was low-pass filtered (60 Hz, 8 pole Butterworth filter).
429 Features extracted from the signals including amplitude, onset, offset, and length of tic related
430 events. LFP onset was defined using a threshold crossing technique, with thresholds being

431 computed from the mean of the peak amplitude. The EMG and audio signals were processed in a
 432 similar manner. Behavioral events were also identified using a frame-by-frame video analysis
 433 and aligned to EMG or audio activity to exclude non-relevant behavioral events, as described
 434 previously (McCairn et al. 2009; McCairn et al. 2012). The time stamps from the acquired signal
 435 were used to construct perievent histograms. Regularity analysis of tics and spike discharges was
 436 computed as CV of the expression rate of the events. Instantaneous CV was computed with 4s
 437 bins and limited to experimental sessions in which clear abnormal behaviors were expressed. In
 438 order to visualize the mean CV the bins derived from the instantaneous CV were randomly
 439 shuffled to remove the large instantaneous variations in the CV. All the statistical procedures
 440 were carried out using Student's t-test.

441 To investigate why vocalization occurred without LFP spikes, interactions of the recorded
 442 regions were examined by analyzing the phase-phase coupling of the individual LFP signals
 443 (Belluscio et al. 2012). This approach was chosen because of its independence from the power of
 444 the LFP signals. Other approaches, such as coherence analysis, are affected by not only the phase,
 445 but also the power of the recorded signals. The sole detection of phase covariance in this case
 446 was appropriate for the evaluation of synchronization, since elevation of power in specific ranges
 447 of frequency was observed for tic conditions (Figure 5A). The coupling condition reads:

$$448 \quad |\Delta\text{phase}| < \text{const}, \quad \text{where } \Delta\text{phase} = \phi_1(t) - \phi_2(t),$$

449 $\phi_1(t)$ and $\phi_2(t)$ are phases of two independent LFP signals from discrete brain regions, e.g.,
 450 NAc and ACC. The distribution of Δphase over time deviates from a uniform distribution if the
 451 two signals oscillate around some constant value. Radial distance of the distribution was used to
 452 quantify the strength of the phase-phase coupling, with $r = 0$ for uniform, and $r = 1$ for a perfect

453 unimodal distribution. In this paper, we refer to this radial distance as *the phase-phase coupling*
454 *strength* (PPCS). For calculating PPCS, LFP segments were first extracted where vocalization
455 occurred with no apparent LFP spikes in each recording session of (NAc : ACC) and (NAc :
456 M1) pairs. Δ phase was then computed for any possible combinations of two frequencies
457 ranging from 5 to 40 Hz with 2 Hz steps. The phase of a given frequency was estimated by
458 performing a Hilbert transform on the band pass filtered LFP signals (2-pass least-square finite
459 impulse response filter). Finally, PPCS was derived from Δ phase averaged out across sessions,
460 minus the same calculation derived from the control condition, where sham events were used to
461 construct comparable data segments, this resulted in a 23x23 matrix for each pair of the
462 recording sites (Figure 5B and 5C). To test for significant differences in the PPCS of specific
463 frequency bands, the population PPCS was calculated and compared to the equivalent
464 frequency range from the control data using Student's t-test (Figure 5D).

465

466

467 **Author Contribution Statement:**

468 K.W.M and Y.N contributed equally to this work. K.W.M, M.M, M.T, T.M, M.I, designed the
469 vocal tic/ PET study. K.W.M, A.I, M.I, designed the motor tic study. Y.N, Y.H, E.K, T.S, TM,
470 K.W.M, M.M, M.I, performed the PET procedures and data analysis. K.W.M, M.I, Y.H, Y.N,
471 M.M, T.M, performed electrophysiological studies. K.W.M, T.N, J.Y.L analyzed all behavioral
472 and electrophysiological data. All authors contributed to writing and editing the manuscript.

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474

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488 conflict of interests.

489

490 **Figure Legends**

491

492 **Fig. 1. Striatal disinhibition causes vocal tics and motor tics in a site-specific manner**

493 (A) An example of vocal tic expression following bicuculline injection in the NAc. The upper
494 trace shows a 60-s record of audio stream. The lower trace shows a magnification of a single
495 vocal tic event with red numbers corresponding to the video frames below. (B) An example of
496 orofacial tic expression following bicuculline injection in the dorsolateral putamen. The upper
497 trace shows a 60-s record of zygomaticus major muscle activity. The lower trace shows a
498 magnification of a single motor tic event. (C) A spectrographic analysis of a vocal tic, which was
499 best described as a grunt. (D) Anatomical locations in which bicuculline injections caused vocal
500 tics (left) and motor tics (right). AC, anterior commissure.

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503

504 **Fig. 2. Temporal dynamics of behavioral and LFP spike expressions characterize vocal and**
505 **motor tics**

506 (A) An example of time-varying characteristic of inter-tic intervals following bicuculline
507 injection in the NAc (time = 0). Vocal tics tended to occur every 2-4 s in the first half of the
508 record. Prob, probability. (B) An example of time-varying characteristic of inter-tic intervals
509 following bicuculline injection in the putamen. Motor tics tended to occur every 1-2 s. (C) Time-
510 dependent changes of actual (broken lines) and shuffled (solid lines) mean CV for vocal tics
511 (black) and motor tics (red). CV was calculated every 4 s. (D) An example of time-varying
512 characteristic of inter-LFP-spike intervals following bicuculline injection in the NAc. (E) An
513 example of time-varying characteristic of inter-LFP-spike intervals following bicuculline
514 injection in the putamen. (F) Time-dependent changes of actual (broken lines) and shuffled (solid
515 lines) mean CV for ACC LFP spikes (black) and M1 LFP spikes (red). CV was calculated every
516 4 s.

517

518 **Fig. 3. PET imaging reveals contrasting involvement of cortico-subcortical structures**
519 **between vocal and motor tics**

520 Increased rCBF following NAc injection contrasted with putaminal injection (Vocal > Motor,
521 *top*), and increased rCBF following putaminal injection contrasted with NAc injection (Motor >
522 Vocal, *middle*). The final panel on each row shows the activation profile laid onto sagittal
523 sections to illustrate the extent of ACC activation for each experimental condition. Each coronal
524 section was obtained at the rostrocaudal level indicated by a corresponding blue line and lettering
525 below.

526

527 **Fig. 4. Electrophysiological recording reveals contrasting spatiotemporal dynamics of LFP**
528 **spikes between vocal and motor tics**

529 (A) An example of LFP spike records following bicuculline injection in the NAc. Note large LFP
530 spikes in the ACC. The number of vocal tics (Audio) is smaller than the number of LFP spikes.
531 (B) Another example of LFP spike records following bicuculline injection in the NAc. Vocal tics
532 could occasionally occur without LFP spikes (gray shading). (C) Showing the mean PSD of the
533 vocal tics with and without LFP spikes, note the similarity in waveform profile and amplitude
534 (D) An example of LFP spike records following bicuculline injection in the putamen. Note large
535 LFP spikes in the M1. M1 LFP spikes show a 1:1 relationship to motor tics (EMG). (E) Spike-
536 triggered averaging of EMG records (mean \pm SEM). This analysis used LFP spikes in the
537 putamen as triggers. (F) Spike-triggered averaging of audio records. This analysis used LFP
538 spikes in the NAc as triggers. Note the existence of two peaks one of which preceded LFP spike
539 onset (time = 0).

540

541 **Fig. 5. Spectral and phase-phase coupling analysis of LFP data during vocal tics**

542 (A) Showing the power spectral density (PSD) of LFP signals acquired during the pre-injection
543 (dashed lines) and vocal tic (solid lines) phases in the NAc (red), ACC (yellow) and M1 (blue).
544 Note the increase of power in the PSD in the alpha (7 - 12 Hz) range during the vocal tic
545 condition. (B) (C) Phase-phase coupling analysis performed on LFP data derived from (NAc :
546 ACC) and (NAc : M1) pairings in periods when vocal tics were observed without any
547 corresponding LFP spikes. The plots show the PPCS interaction between frequency ranges after

548 subtraction with equivalent phase-phase coupling analysis conducted in the pre-injection
549 condition. Note the elevated (red shading) signal in the alpha (7 - 12 Hz) frequency range. (D)
550 Bar-plot showing the mean PPCS calculation for each condition, significant differences between
551 conditions is indicated by an asterisk.

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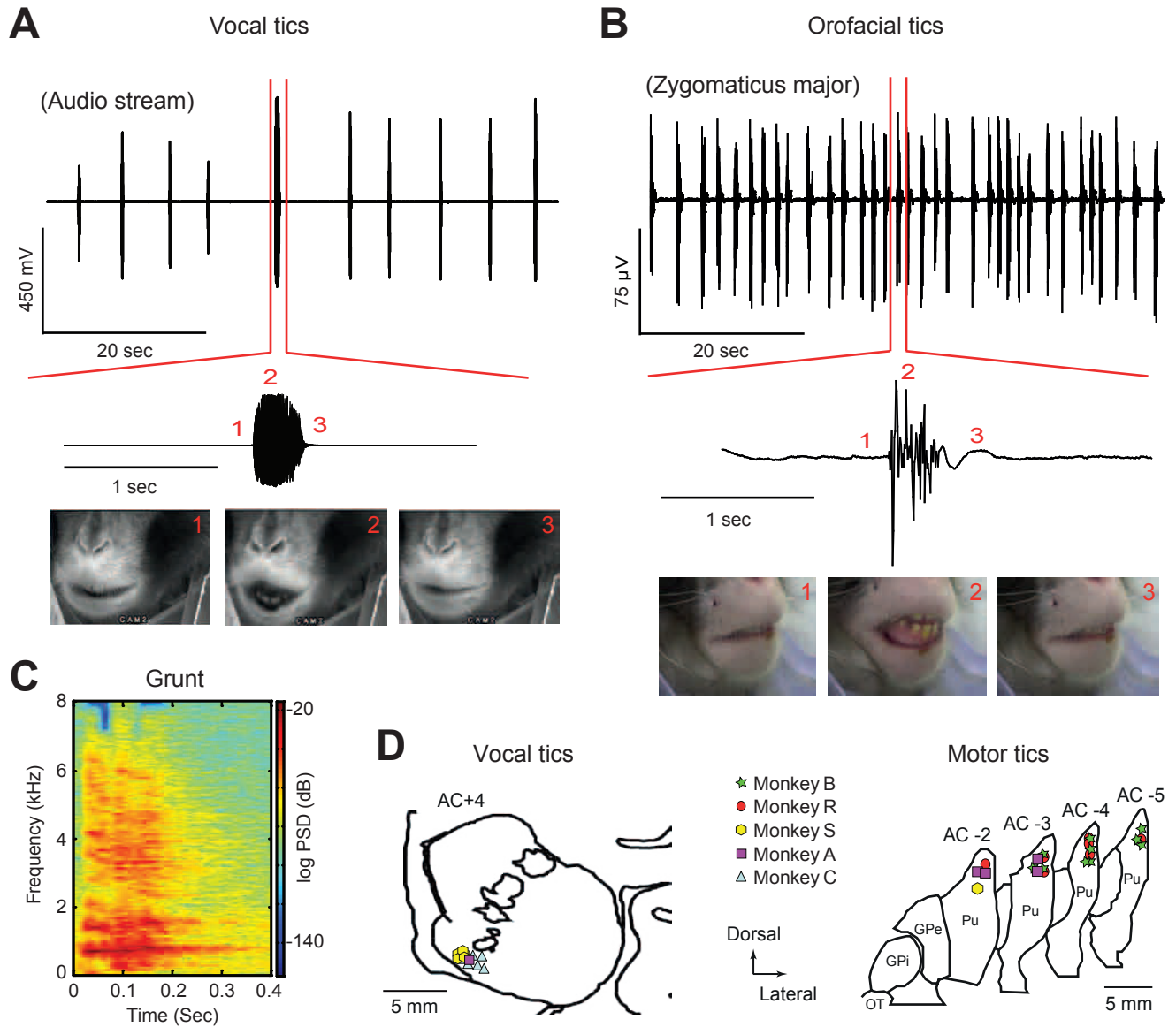
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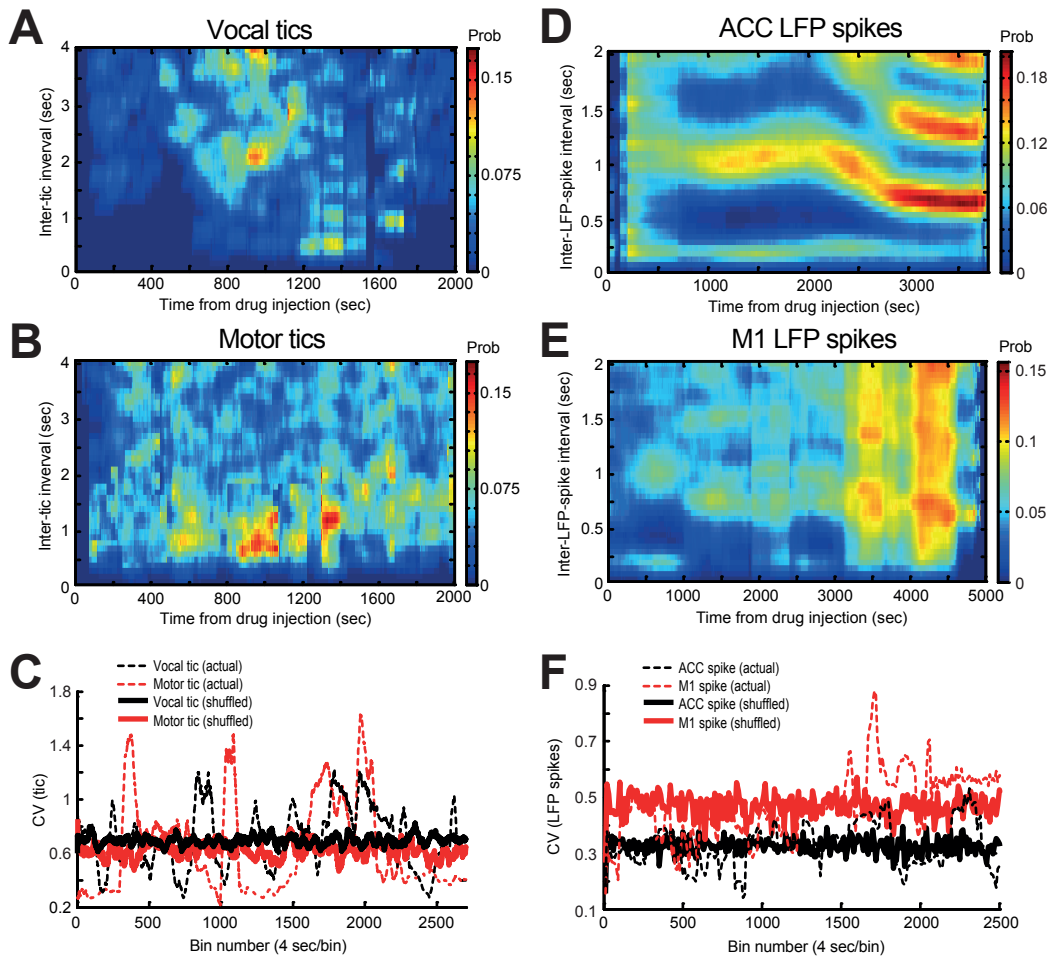
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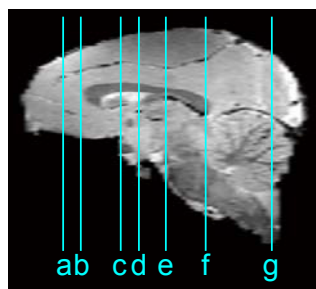
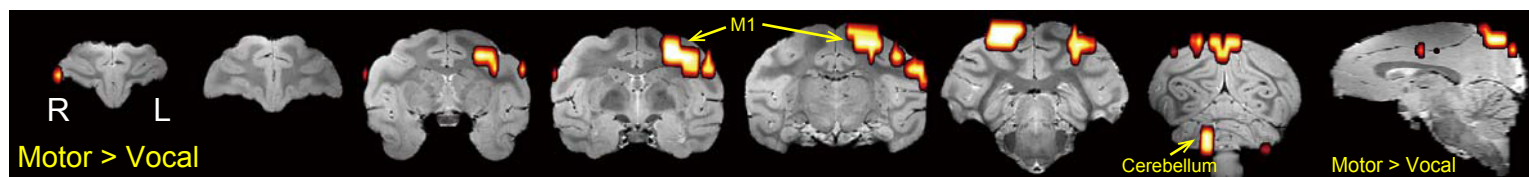
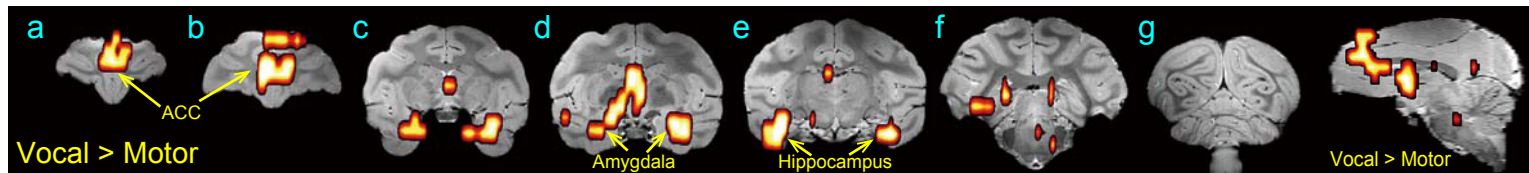
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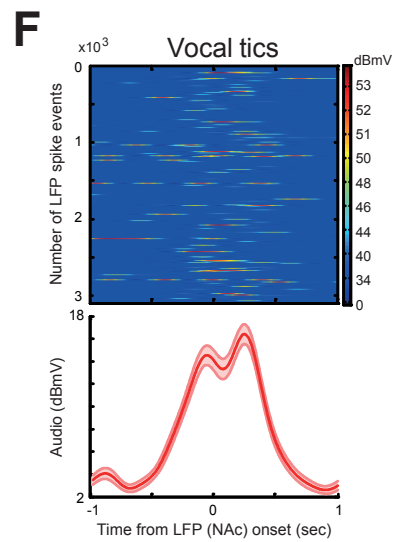
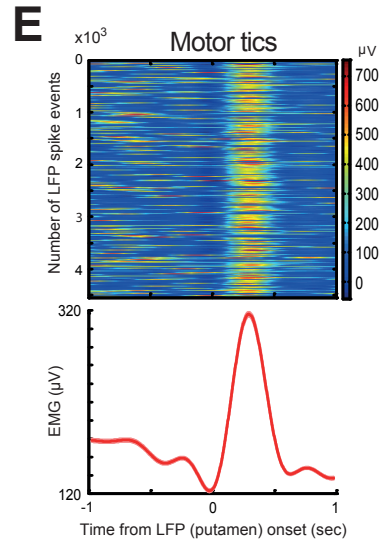
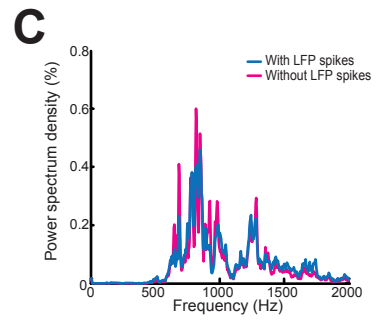
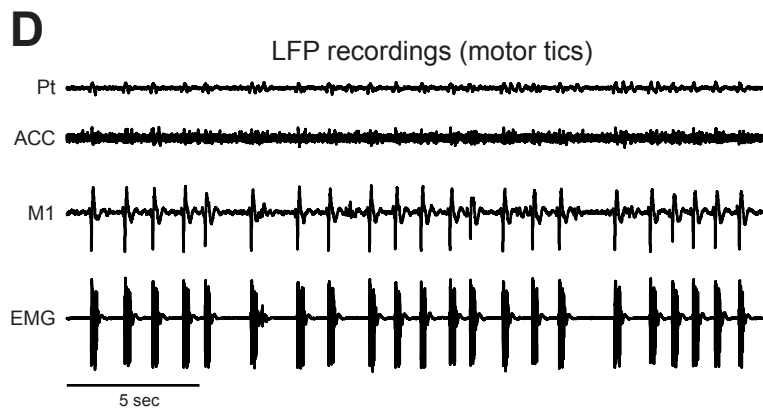
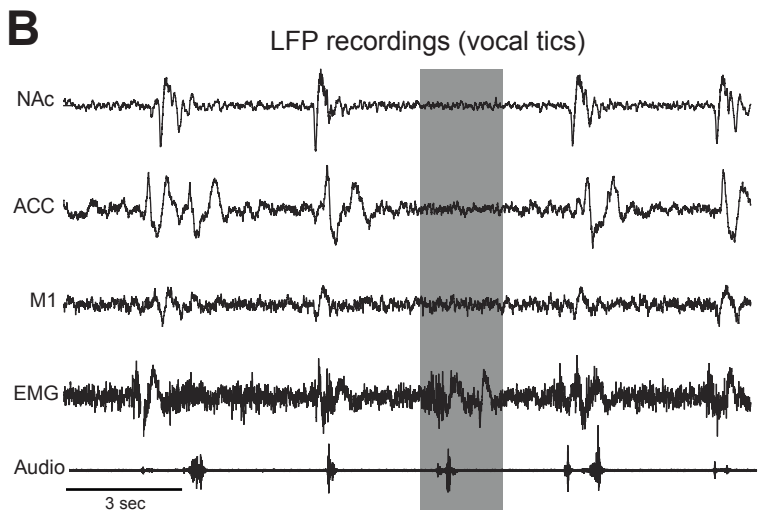
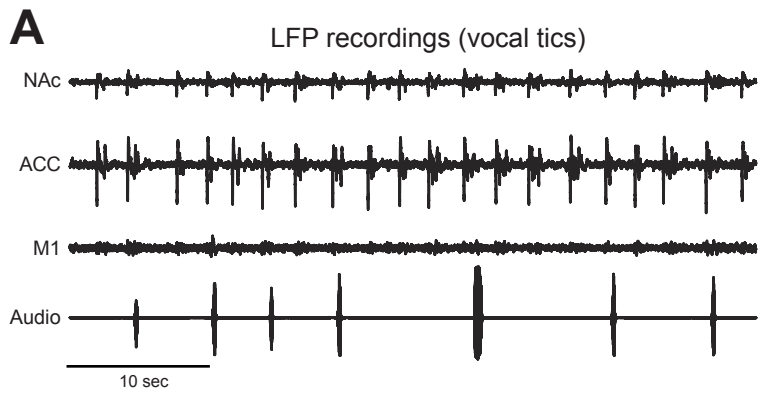
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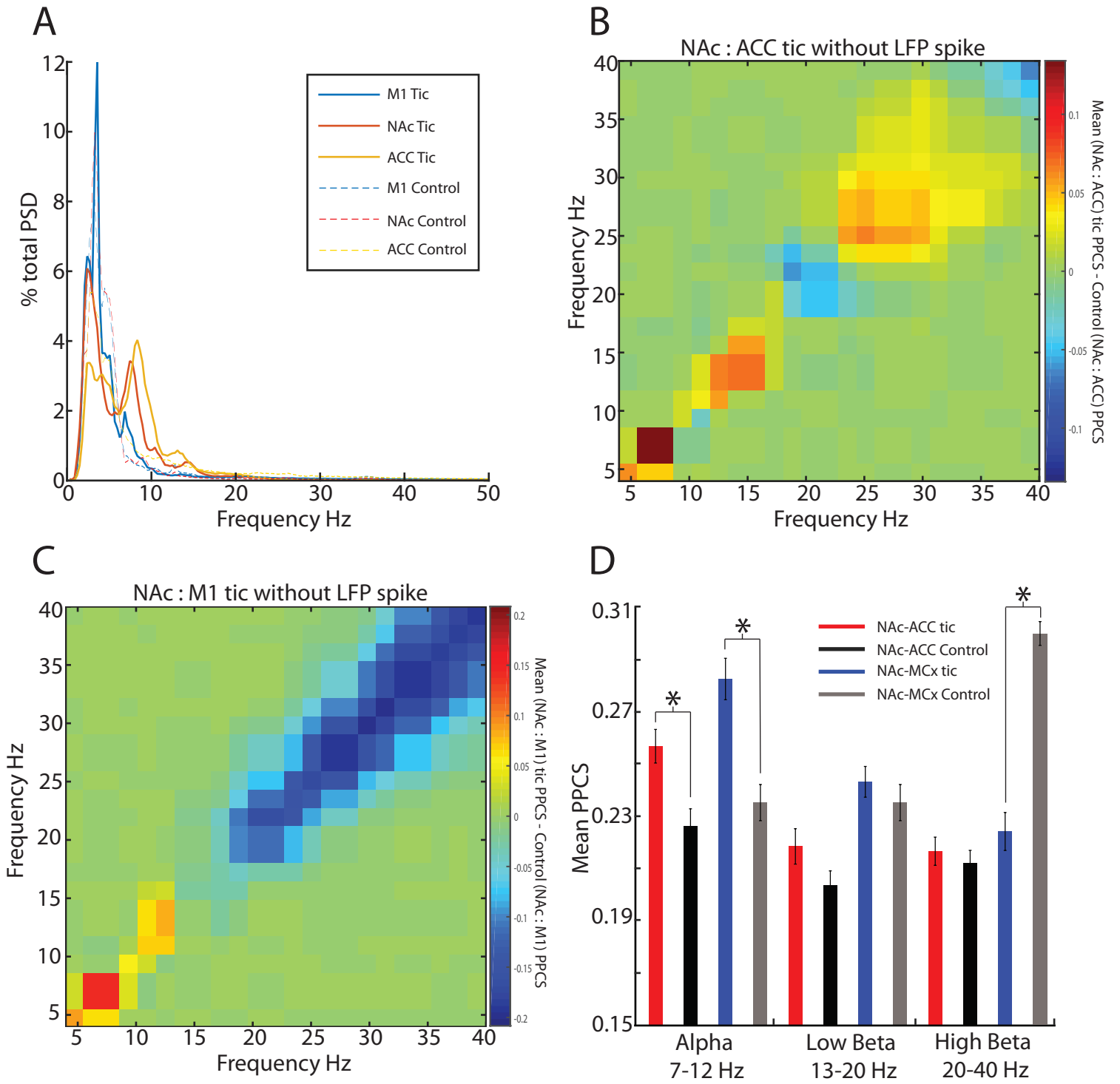
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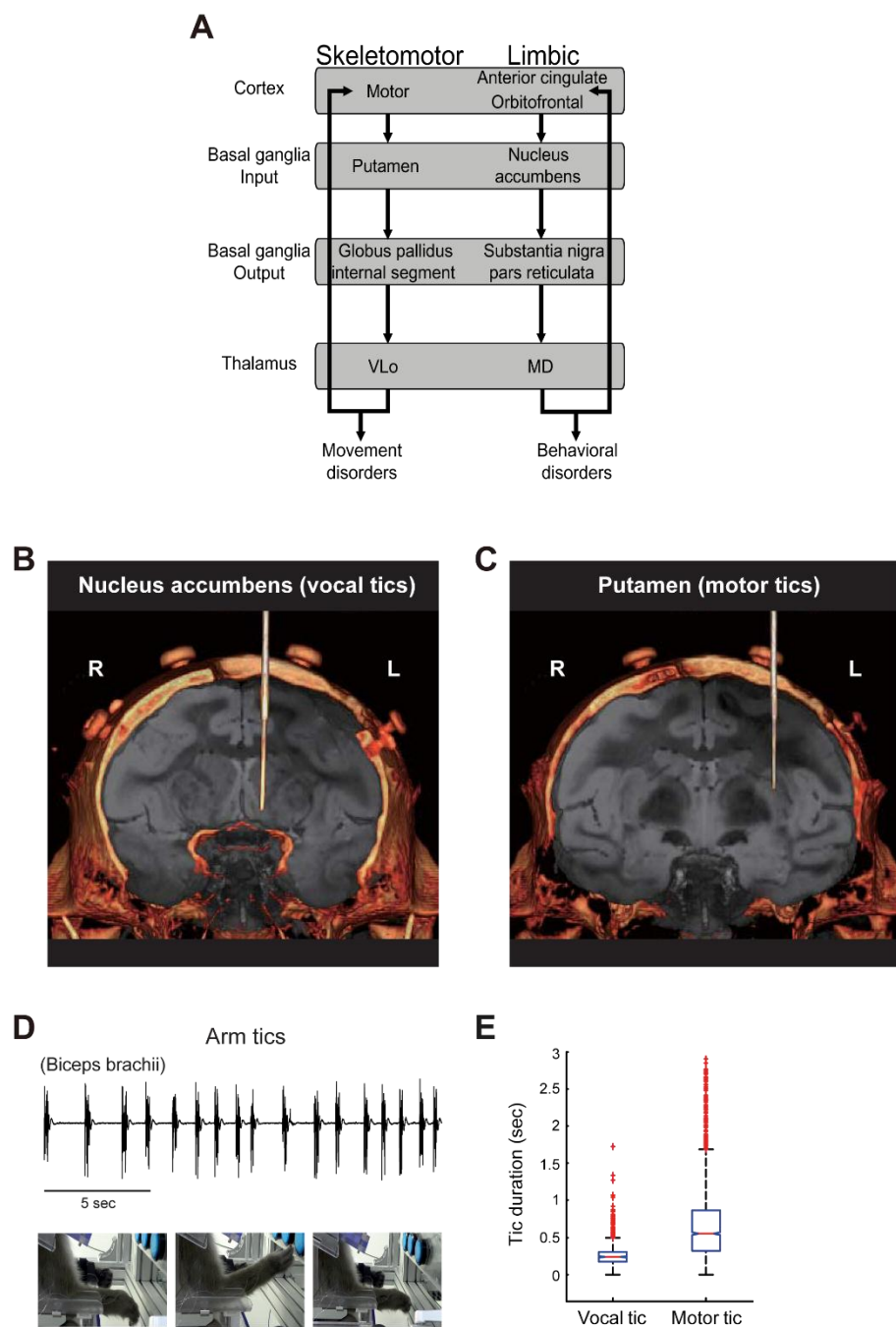


Rostral ← → Caudal



Figure





Supplemental Figure 1 (related to Figure 1) McCairn et al.

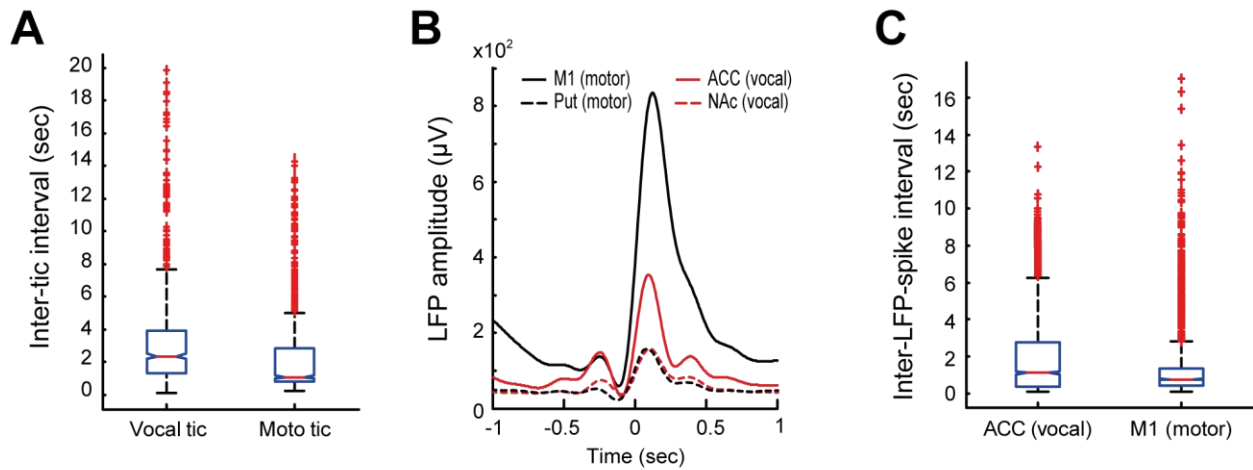
Figure S1 (related to Figure 1). Anatomical and behavioral characterizations of vocal and motor tics.

(A) Shown are the key nodes in the sensorimotor and limbic networks, microinjection of

bicuculline was targeted to the basal ganglia input nodes – putamen and nucleus accumbens.

VLo = Ventral lateral oralis, MD = Medial Dorsalis.

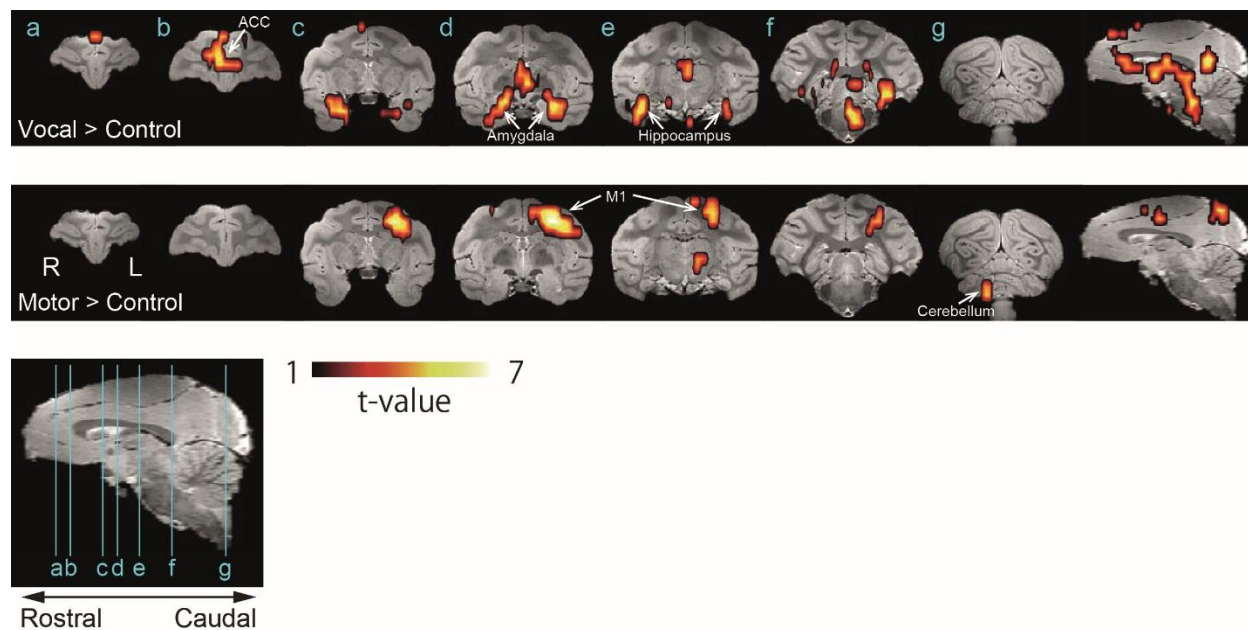
(B) A fusion image of MRI/CT showing the location of the injection cannula targeted to the NAc for inducing vocal tics (4 mm rostral to the anterior commissure). (C) A fusion image of MRI/CT showing the location of the injection cannula targeted to the dorsolateral putamen for inducing motor tics (2 mm caudal to the anterior commissure). (D) An example of arm tic expression following bicuculline injection in the dorsolateral putamen. The upper trace shows a 20-s record of biceps brachii muscle activity. The lower trace shows a sequence of video frames capturing a single tic event. (E) Comparison of individual tic length between vocal tics and motor tics. The horizontal ends of each box indicate upper and lower quartile values; whiskers are extended to the most extreme value 1.5 fold the interquartile range; notches in the side of the boxes display the 95 % confidence interval; and outliers are displayed as + symbols.



Supplemental Figure 2 (related to Figure 2) McCairn et al.

Figure S2 (related to Figure 2). Comparison of behavioral and LFP spike dynamics between vocal tics and motor tics.

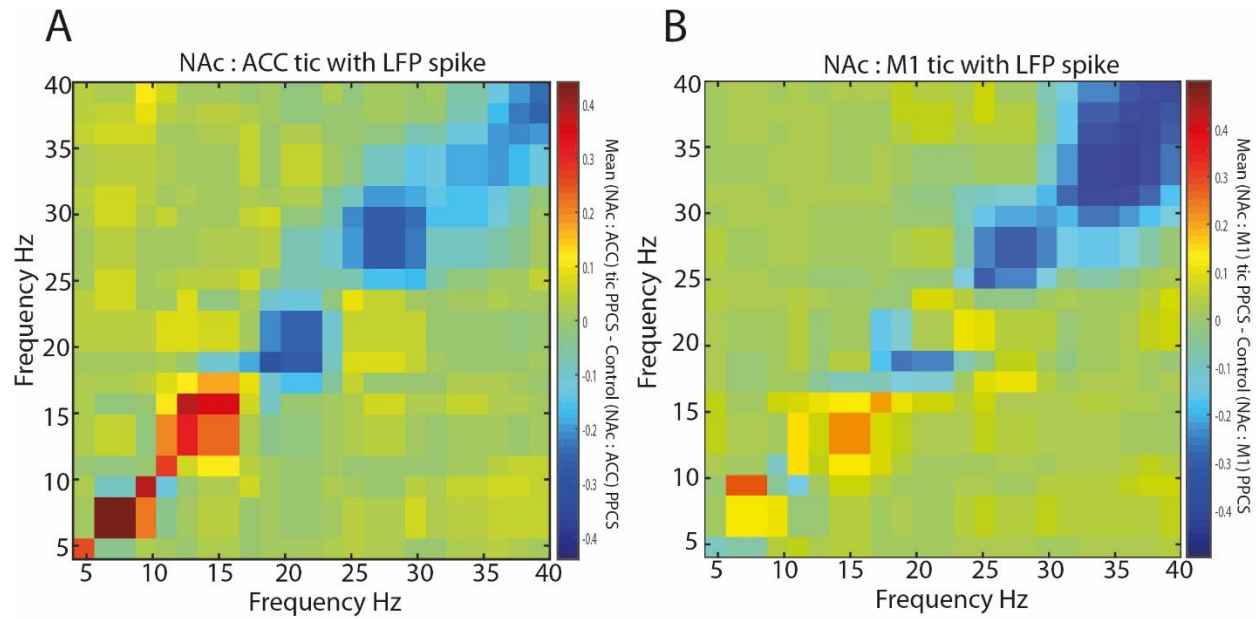
(A) Comparison of inter-tic intervals between vocal tics and motor tics. Same conventions as in Figure S1D. (B) Comparison of LFP size between M1, putamen (Put), ACC, and NAc. (C) Comparison of inter-LFP-spike intervals between vocal tics and motor tics. Same conventions as in Figure S1D.



Supplemental Figure 3 (related to Figure 3) McCairn et al.

Figure S3 (related to Figure 3). rCBF increases during vocal tics and motor tics contrasted with their baseline control.

Increased rCBF following NAc injection contrasted with putaminal injection (Vocal > Control, *top*), and increased rCBF following putaminal injection contrasted with NAc injection (Motor > Control, *middle*). The final panel on each row shows the activation profile laid onto sagittal sections to illustrate the extent of ACC activation for each experimental condition. Each coronal section was obtained at the rostrocaudal level indicated by a corresponding blue line and lettering below.



Supplemental Figure 4 (related to Figure 5) McCairn et al.

Figure S4 (related to Figure 5). Analysis of phase-phase coupling associated with vocal tics and LFP spikes

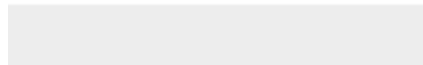
(A) and (B) phase-phase coupling analysis performed on LFP spikes that are present during vocal tics between NAc, ACC and M1. Note the increased coupling strength in the alpha (7- 12 Hz) range.



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