Simultaneous analysis of the LFP and spiking activity reveals essential components of a visuomotor transformation in the frontal eye field

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Edited by Thomas D. Albright, The Salk Institute for Biological Studies, La Jolla, CA, and approved May 8, 2017 (received for review March 6, 2017)

The frontal eye field (FEF) is a key brain region to study visuomotor transformations because the primary input to FEF is visual in nature, whereas its output reflects the planning of behaviorally relevant saccadic eye movements. In this study, we used a memory-guided saccade task to temporally dissociate the visual epoch from the saccadic epoch through a delay epoch, and used the local field potential (LFP) along with simultaneously recorded spike data to study the visuomotor transformation process. We showed that visual latency of the LFP preceded spiking activity in the visual epoch, whereas spiking activity preceded LFP activity in the saccade epoch. We also found a spatially tuned elevation in gamma band activity (30–70 Hz), but not in the corresponding spiking activity, only during the delay epoch, whose activity predicted saccade reaction times and the cells’ saccade tuning. In contrast, beta band activity (13–30 Hz) showed a non-spatially selective suppression during the saccade epoch. Taken together, these results suggest that motor plans leading to saccades may be generated internally within the FEF from local activity represented by gamma activity.

Significance

Converting a visual input into a motor output is a fundamental computation that nervous systems have evolved to perform. In the context of saccadic eye movements, several brain areas have been identified that exhibit the effect of visuomotor computations. Nevertheless, because of dense interconnectivity between these areas, the contribution of a particular brain area to the visuomotor transformation process has not been clarified. By simultaneously recording from the local field potential (LFP) that is thought to reflect input to an area, and spiking activity that reflects its output activity, we showed that frontal eye field neurons perform the necessary visuomotor transformation to generate a saccade plan internally. We also identify specific components of the LFP such as gamma oscillations that may enable such a visual-to-motor transformation.

Author contributions: N.S. and A.M. conceived the study; D.B. collected data; N.S. analyzed data; and N.S., D.B., and A.M. wrote the paper.

The authors declare no conflict of interest.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1703809114/-/DCSupplemental.

www.pnas.org/cgi/doi/10.1073/pnas.1703809114

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Results
To compare the timing of visual and saccadic responses in the LFP and simultaneously recorded spikes, we trained two macaque monkeys to perform a memory-guided saccade task, to dissociate the visual and saccadic epochs (SI Materials and Methods and Fig. 1). Fig. 2 shows an example neural signal and simultaneously recorded LFP in one representative site having both visual and saccadic responses as a function of eight stimulus/saccade positions. The sensory-motor responses seen in both LFP and spikes were also spatially tuned (Fig. 2, Inset).

Comparison of LFP and Spike Timing in the Visual Epoch. In the first stage of a saccade plan, FEF receives visual information as an input from many brain regions through the dorsal and the ventral pathways. Earlier studies have shown that the LFP signals in FEF have visual response latencies (VRLs) significantly earlier than spikes, suggesting that the LFP reflected the visual input to FEF (1, 5). In this study, we first investigated the temporal relationship between the LFP and spikes in the visual epoch to confirm this result.

We calculated the VRL of LFP and spikes by performing a \( t \) test between the baseline and the signals in the visual epoch (SI Materials and Methods). Fig. S1 shows the result of this analysis for an example LFP site (Fig. S1A) and a simultaneously recorded neuron (Fig. S1B). For all 66 recorded sites, we found that the LFP showed VRL earlier than spikes (\( n = 66; P < 10^{-17} \)), albeit with different latency differences between them. The VRL for the population of recorded LFP sites was 58.9 ± 1.6 ms and that of the spikes was 78.4 ± 1.2 ms. The mean difference between LFP and spike VRL was \(-19.5 ± 1.4 \text{ ms} \) (Fig. S1C). This result suggests that the LFP has information regarding the location of the visual stimulus significantly earlier than the spikes in FEF, supporting previous studies (1, 28).

Comparison of LFP and Spike Timing in the Saccade Epoch. We calculated the time of saccade plan onset (SPO) as the first point before the start of the saccade when the signal (LFP/spike) differed from baseline (SI Materials and Methods). Fig. 3 shows the result of this analysis for an example neuron (Fig. 3A) and the simultaneously recorded LFP (Fig. 3B). The SPO_{SPK} was significantly earlier than the SPO_{LFP} (\( n = 66; P < 10^{-21} \)) in all the sessions. Also, whereas the SPO_{SPK} showed a distributed range spanning a significant amount of the presaccadic epoch, the SPO_{LFP} was distributed in a narrow epoch close to the start of the saccade. The mean SPO_{SPK} for the population was \(-215.0 ± 7.0 \text{ ms} \) and the mean SPO_{LFP} was \(-84.4 ± 5.6 \text{ ms} \) before the start of the saccade, with a mean difference (\( \Delta \text{SPO} \)) of \(-130.6 ± 9.0 \text{ ms} \) (Fig. 3C).

We also calculated the saccade plan specification (SPS) time as the first time point before the start of the saccade when the signals (LFP/spikes) in at least one of the eight spatial positions differed from others (SI Materials and Methods). Fig. 4 shows the result of this analysis for an example neuron (Fig. 4A) and simultaneously recorded LFP (Fig. 4B). SPS_{SPK} was earlier than SPS_{LFP} for all the sessions. For the population, the mean SPS_{SPK} was \(-141.9 ± 6.2 \text{ ms} \) and the mean SPS_{LFP} was \(3.2 ± 4.7 \text{ ms} \) before the start of the saccade, with a difference (\( \Delta \text{SPS} \)) of \(-145.1 ± 8.0 \text{ ms} \) (Fig. 4C).

SPS_{SPK} and SPS_{LFP} showed a similar trend as the SPO_{SPK} and SPO_{LFP} times, but shifted in time (Fig. 5A). The LFP showed an onset only 57.5 ± 8.3 ms after the spikes specified the saccade plan. Spikes specified the saccade direction approximately 73.1 ± 5.0 ms after they showed onset and the LFP specified the saccade direction approximately 87.6 ± 4.6 ms after their onset, suggesting that the spikes took less time to specify the saccade direction after their onset compared with the LFP (\( P = 0.0148; \) Fig. 5 B and C).

Furthermore, the SPO_{SPK} correlated with the SPS_{SPK} (\( r^2 = 0.7166; P < 10^{-11} \)) and so did their LFP counterparts (\( r^2 = 0.6046; P < 10^{-8} \)); however, and more importantly, the SPO_{SPK} – SPS_{SPK} latency did not correlate with the SPO_{LFP} – SPS_{LFP} latency (\( r^2 = 0.134; P = 0.2830 \)). Further, neither the SPO_{SPK} correlated with the SPO_{LFP} (\( r^2 = 0.036; P = 0.9771 \)) nor was the SPS_{SPK} correlated with the SPS_{LFP} (\( r^2 = -0.0526; P = 0.6747 \)). Finally, the SPO_{LFP} did not correlate with the SPS_{LFP} either (\( r^2 = 0.0357; P = 0.7760 \)), suggesting completely different origins for spikes and LFP in the saccade epoch (Fig. 5D). Taken together, these results suggest that in the saccade epoch, the plan for onset of saccade and the direction of saccade is specified in spikes significantly earlier than, and independent of, the LFP. We return to this point in our discussion where we suggest possible explanations for our observation.

Characterization of Gamma Band Activity in FEF. We investigated the functional role of two prominent frequency components of LFP, the gamma and the beta bands, in the visuomotor transformation process in FEF. The frequency domain information in the LFP
signal was retrieved by using a Multi Taper analysis to obtain the power of each frequency component as a function of time (SI Materials and Methods). Fig. 6 shows a time-frequency spectrogram constructed from a representative LFP recording signal. The gamma power was spatially tuned in the delay period (Fig. S2) and showed elevated activity in the receptive field (RF) position (Fig. 6A, Left) which sustained in the delay period and returned to baseline in the saccadic epoch (Fig. 6A, Right). This effect, while still present, was less prevalent in the antireceptive field (aRF) position (Fig. 6B).

Fig. 7A shows average z-scored gamma power as a function of time in the RF and aRF positions for the representative LFP site. Gamma showed a significant elevation in activity from baseline (0.03 ± 0.03) only in the delay epoch (0.29 ± 0.06; n = 58, P = 0.001), after which it returned to the baseline and, hence, showed no significant modulation in the saccadic epoch (0.20 ± 0.09; P = 0.129; Fig. 7B). The gamma activity was also spatially tuned in the delay epoch (RF: 0.44 ± 0.07, aRF: 0.15 ± 0.06; P = 0.01; Fig. 7C). Interestingly, although the rise time for gamma, from the baseline, in RF was significantly earlier than in aRF (RF: 143.2 ± 13.9 ms, aRF: 233.8 ± 18.9 ms; P = 0.008), the peak time was comparable (RF: 257.7 ± 6.6 ms, aRF: 243.9 ± 7.9 ms; P = 0.402). Hence, the time when gamma differed between RF and aRF (154.3 ± 17.3 ms) was close to the gamma rise time in the RF position (Fig. 7D).

The above results characterize gamma activity as a spatially modulated oscillation in the delay epoch. We asked whether this activity profile of gamma could be linked with the sensory-motor integration processes (23, 24), especially because of its timing and spatial tuning properties. Because the neurons that were recorded simultaneously with the LFP also showed spatial tuning in both visual and saccadic epochs, we asked whether the gamma tuning was correlated with either of these neuronal tuning properties. Hence, we plotted the Spearman rho values of pairwise correlations between the saccade tuning and gamma tuning (ordinate) against the pairwise correlations of visual tuning and gamma tuning (abscissa), for each neuron. We found that the gamma activity’s tuning was more similar to the saccade tuning than the visual tuning of the neurons (Fig. 7E). To account for the possibility that the neural firing in the delay period might have an influence on the gamma activity, we also calculated the spike tuning in the memory epoch and correlated that with the gamma activity and found that none of the pairs were significantly correlated (spearman correlation; P > 0.05; Fig. S3A). Taken together, these results suggest that gamma activity could reflect the sensory to motor transformation.

To test this hypothesis further, we asked if gamma in FEF could predict the saccadic reaction times (RTs) of the animal. We calculated a trial-by-trial average of gamma activity in the delay period and estimated the RT as the time of the saccade onset from the “go” signal. We then classified the RT into four quantiles, and we took the first quantile as the “fast” group and the last quantile as the “slow” group to see the effects in the extreme ends. We also binned the average gamma activity based on these two RT groups. We found that the gamma in the delay period could predict the RT, with the effect being stronger in the RF positions (Fig. 7F; RF fast: 0.79 ± 0.05, RF slow: 0.47 ± 0.06; n = 58, P < 10−5; aRF fast: 0.51 ± 0.06, aRF slow: 0.40 ± 0.06; P < 10−14), but the spiking activity in the same epoch couldn’t (RF, P = 0.8375, aRF, P = 0.3032; Fig. S3B).
Comparison of saccade plan onset and specification times for spikes and LFP. (A, Top) The average firing rate from a representative neuron in each of the eight positions (gray) superimposed with the average firing rate across all eight positions (green). SPO is shown as a solid line, and the SPS is shown as a dashed line. (A, Lower) Same format as Upper, but for LFP from a representative site. All traces were taken from Figs. 3 and 4. (B) The SPO (solid lines) and SPS (dashed lines) for LFP (green) and spikes (blue) for all of the sessions, sorted by SPO. The plot is smoothed for ease of viewing. Upper shows the mean and SEM for the SPO and SPS for LFP and spikes. All traces were taken from Figs. 3 and 4. (C) Pairwise differences between the SPO_ΔPK, SPO_ΔSP (ΔSPK), SPO_ΔLP and SPS_ΔLP. Pairwise differences between the SPO_ΔPK and SPO_ΔPK (ΔSPK) and the SPS_ΔPK and SPS_ΔPK (ΔSPS). Difference between SPO_ΔPK and SPO_ΔPK. The mean and the SIs are shown as superimposed black markers. (D) The SPO and SPS were well correlated within spike and LFP, but not across spike and LFP. Upper Left, scatter plot between the SPS_ΔPK (abscissa) and SPO_ΔPK (ordinate). Regression line is shown as a solid blue line. Upper Middle, similarly for LFP. Upper Right, time lag between SPO and SPS was not correlated between spikes and LFP. Lower, same format as above, but for SPO_ΔPK vs. SPO_ΔPK onset (Left); SPS_ΔPK vs. SPS_ΔPK (Middle); SPO_ΔLP vs. SPS_ΔPK (Right).

Fig. 5). Although beta activity showed a distinct modulation in activity during the saccade preparation epoch, it lacked spatial tuning, did not predict the RT, and was temporally proximal to the saccade onset.

Discussion

By comparing the time course and frequency components of the LFP relative to spiking activity of FEF neurons, we report two main findings. First, the LFP showed visually evoked responses significantly earlier than the spikes (Fig. S1), but the spikes had information about the planned saccade (Fig. 3) and its direction (Fig. 4) significantly earlier than the LFP (Fig. 5). Second, gamma activity showed elevated activity with spatial selectivity in the delay epoch, after the visual stimulus presentation and well before the saccade epoch, with a tuning more similar to the neural saccadic tuning than the visual tuning. Gamma activity also predicted the RT, suggesting that it represents an important aspect of the local computation that represents the visuomotor transformation occurring within FEF (Fig. 7). In contrast, beta activity showed a nondirectionally selective suppression in activity just before saccade initiation (Fig. 8). Here, we discuss some important implications of our results.

Input Characteristics of FEF. The VRL that we report is in agreement with the previously reported values by other groups (ref. 1; LFP, early: 51.7 ± 0.5 ms, late: 61.5 ± 0.8 ms; spike, early: 65.0 ± 3.3 ms, late: 79.0 ± 2.9 ms. Ref. 28; LFP: 56.5 ms and spike: 68.5 ms). We report a VRL of 58.9 ± 1.6 ms in LFP and 78.43 ± 1.16 ms in spikes (Table S1), which suggests that the LFP has information regarding the location of the visual stimulus significantly earlier than the spikes in FEF, supporting previous studies (1, 28).

The Role of FEF in Saccade Planning. In this study, we show that although the LFP in FEF had an earlier visually evoked response in the saccade epoch, the spiking activity of FEF neurons possessed information about the planning of saccade and its direction earlier than the LFP by ~130 ms for SPO and ~145 ms for SPS across the two monkeys (Table S1). This reported relationship between LFP and spikes was not affected by the potential effects due to LFP or spike filtering (SI Materials and Methods and Fig. S4). Interestingly, saccade selectivity in LFP developed within a temporally narrow epoch, very close to the saccade execution time. Hence, if we interpret LFP selectivity to represent input and spiking selectivity to represent output, we infer that FEF generates saccade plans internally and may represent the first step where the critical visuomotor transformation occurs. We report markers for two phases of saccadic computation: SPO time, when the global plan for a saccade is initiated, and SPS time, when the direction of the saccade being planned is selected. We further showed that the relationship between spikes and LFP was similar in both these phases of computation.

In this context, it is interesting to note that prior studies have shown that the lateral intraparietal area (LIP), which provides prominent input to FEF and also possesses cells with visuomotor properties, shows the converse relationship between LFP and spiking activity, where LFP has earlier and more robust information regarding the planning and execution of saccades than the spikes (21). These evidences taken together suggest that saccade plans are generated in FEF internally and relayed to LIP through their reciprocal feedback connections. These results are consistent with the notion that LIP is primarily a visual area playing an active role in visual selection but a more passive role in the visuomotor transformation process. Consistent with this interpretation, saccade countermarching or active online cancellation of saccades is readily observed in the activity of movement-related cells in the FEF but not in LIP (29).

If the SPS_ΔPK time represents an intrinsic visuomotor transformation occurring within FEF, then what does the ensuing SPS_ΔLP reflect? Because electrical stimulation of FEF requires at least 30–45 ms to elicit a saccade (30), one can assume that a signal, in FEF, showing a SPO or SPS less than 30–45 ms before saccade might not play a potential role in saccade generation. In this study, we report a SPO_ΔLP of ~84.4 ± 5.6 ms and a SPS_ΔLP of 3.2 ± 4.7 ms relative to saccade onset, supporting our argument.

Fig. 6. Time-frequency spectrogram for a representative LFP site. (A) Time-frequency spectrogram for the RF position aligned to stimulus onset (Left) and saccade onset (Right) showing an increase in gamma (30–70 Hz) power in the delay epoch and a decrease in beta (13–30 Hz) power before saccade. (B) Same format as A above, but for arRF, showing a low level of gamma increase and no change in beta decrease.
Visual selection is an important component of the visuomotor transformation process, necessary for planning of relevant saccades. It is also considered as the link between perceptual processes and action or execution (34). Monosov et al. showed that FEF neurons have visual selection times (105–133 ms) significantly earlier than the LFP (134–152 ms) and that the FEF neurons were the functionally active units that performed this computation from their visual input (LFP) from other brain regions. Hence, the later selection time noted in the LFP could be a reflection of local computation in FEF. Because we used a memory-guided saccade task in our study, it did not involve classical target selection due to the absence of distractors. Nevertheless, the time of gamma increase we report might be a signature of this covert visual attention process and goal selection for the upcoming saccade.

Whereas the spatial selectivity of gamma activity during the delay period (23, 24) implies a role in working memory of stimulus location, it is typically thought to reflect sensory memory, and like visual selection, is not expected to be correlated with RT (36). However, consistent with previous work, we showed that gamma activity, but not the corresponding spiking activity, was correlated with RT (23, 24). Additionally, the gamma activity in the delay epoch was more closely tuned to the saccadic tuning than the visual tuning of the cell. These results suggest that gamma oscillations may reflect intracortical processing within FEF that aids in transformation of sensory information into a saccade plan.

**Beta Activity Represents Undifferentiated Neural Activity.** Beta suppression has been considered as an “undifferentiated reflection of neural activity” (26) because the amplitude of suppression is not clearly modulated by motor parameters like direction (36), speed (26), or duration (37). However, in oculomotor regions, it gets modulated in an epoch close to the initiation of the saccade but the modulation characteristics itself might differ between different cortical regions, from being a spatially invariant suppression of activity in regions like LIP before a saccade (21, 38) or a spatially selective elevation of activity in regions like the posterior parietal cortex before a coordinated reach and saccade (39). We investigated beta modulation in the FEF and characterized the properties of beta band activity in the visual, delay, and the saccade epochs to check for any functional significance during the visuomotor transformation process.

In contrast to gamma activity, beta band activity was not spatially tuned and showed a suppression in activity close to saccade onset but did not correlate with RT. Because the beta band activity represented a gradual suppression in power, starting before the eye movement and was just not a transient

**Gamma Band Activity Reflects the Visuomotor Transformation.** Previous studies in LIP have shown that in the delay epoch, a spatially tuned elevation in activity in the gamma band (30–70 Hz) might contribute to the maintenance of “memory fields,” which are important in working memory (21), whereas other studies have suggested that gamma activity could be involved in the sensory-motor integration process (23, 24). Our results indicate that these two roles might not be mutually exclusive. The gamma activity raised in power in a time frame that was much later than the initial visual response period in sensory-motor regions including FEF (33) and LIP (21) but also much earlier than the saccade epoch and, therefore, may represent an important intermediate step of visual selection that precedes saccades.

**Fig. 7.** Gamma band properties. (A) z-scored gamma power of a representative LFP site for RF (yellow) and aRF (brown) positions, aligned to stimulus onset (Left) and saccade onset (Right). Shading, standard deviation. (B) The average peak z-scored gamma power for the population of recorded LFP sites in the delay epoch, 150–350 ms after the visual stimulus presentation and in the saccade epoch, ~300 to 100 ms from saccade onset, t test P < 10−5. (C) The average peak z-scored gamma power for the population of recorded LFP sites in the memory period in the RF (yellow) and aRF (brown). (D) Onset time for gamma power in RF (1, yellow) and in aRF (1, brown). The dotted line shows the first time when the gamma power in RF significantly differed from the gamma power in aRF position. Peak time for gamma power in RF (2, yellow) and in aRF (2, brown). (E) A scatter plot between the r2 values obtained from correlating gamma tuning with visual tuning of spikes (x axis) and correlating gamma tuning with the saccadic tuning of spikes (y axis) showing that gamma tuning is more linked with saccadic tuning. The thin solid line is the line of unity. (F) Gamma activity in the RF (Left) and the aRF (Right) positions for fast (F) and slow (S) RTs.

**Fig. 8.** Beta band properties. (A) Average z-scored beta power aligned to stimulus onset (Left) and saccade onset (Right) showing a decrease in beta power below the baseline level, during the saccade epoch. Shading: standard deviation. (B) The average peak z-scored beta power for the population of recorded LFP sites in the visual epoch, delay epoch, and the saccade epoch. (C) The average z-scored beta power in all eight positions for the population of recorded LFP sites in the saccade epoch showing no significant tuning. (D) Population average beta activity for fast (F) and slow (S) RTs.
phenomenon revealed solely at the time of saccade execution, we believe that it is unlikely to reflect an EOG signal artifact. Additionally, beta band power started to differ from baseline at a time point later than the saccade specification time of FEF and is therefore unlikely to reflect a direct role in saccade specification, similar to what has been shown in LIP (21, 38). Recent evidence however suggests that beta activity might be a reflection of motor preparation (40) and may be involved in other aspects of motor preparation such as eye-hand coordination (39) or a corollary discharge signal.

Materials and Methods

Two adult monkeys, J (male, Macaca radiata) and G (female, Macaca mulatta) were used for the experiments. They were cared for in accordance with the Committee for the Purpose of Control and Supervision of Experiments of Animals, Government of India. Full details on the task design, physiology, and analysis can be found in the SI Materials and Methods.

ACKNOWLEDGMENTS. We thank Dr. Supratim Ray, Vinay Shihatti, and Dr. Sumitash Jana for their initial help with data analyses. This work was supported by an intensification of Research in High Priority Areas Grant from the Department of Science and Technology, Government of India; a Department of Biotechnology - Indian Institute of Science (DBT-IISc) partnership programme grant; and institutional support from the Ministry of Human Resource Development. N.S. was supported by Kishore Vaigyanik Protsahan Yojana Scholarship awarded by Department of Science and Technology, Government of India. We also thank Prof. Michael E. Goldberg (Columbia University) for intellectual inputs and financial support by National Eye Institute/National Institutes of Health (NEINIH) Grants 1 R01 EY017039-02 and 1P30 EY019007-01; the Mahoney Chair in Brain and Behavior Research of Columbia University; and Environmental Memory (a grant from the Zegar family foundation to N.S.) to present these findings in the Society For Neuroscience meeting, 2016 at San Diego.