Understanding motor cortex function: insights from electrocorticography in the rat brain

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1. Introduction

One of the most crucial questions at the heart of neuroscience is that of the biological origins of intelligence. Nervous systems throughout the animal kingdom produce many complex behaviors, but intelligence *per se* is arguably only found in few species. One might argue that true intelligence only exists in human beings, but many other mammals as well can be said to exhibit intelligent behavior. [3, 13] So what is it about the brains of such species that endows them the gift of intelligence?

One simple answer to this question is the presence of the neocortex, a highly organized brain structure only existent in mammalian brains. [15] Other "intelligent" animals such as reptiles and birds arguably have homologous brain structures, and the evolution of mammalian brains - particularly that of the human brain - has followed a trend of neocorticalization, whereby the proportion of brain volume dedicated to the neocortex is larger in more evolutionarily recent - and presumably more intelligent - brains. [15] Along with classical functional localization studies connecting cortical areas to higher cognitive function [8, 14, 2, 22], these observations indeed suggest that the neocortex may be at the heart of the biological substrates of intelligent behavior.

So what exactly does it do? How does it contribute to the dramatic difference in behavioral complexity between species with and without cortex? Here, we aim to take a small step towards answering these big-picture questions by investigating the motor cortex - the part of the neocortex presumably responsible for the generation of motor movements. [11, 12] The reason for focusing on this particular cortical area is twofold. Firstly, flexible and adaptive motor control is evidently a centerpiece of intelligent behavior. Thus, understanding the role cortex plays in such cognitive processes may help shed light on the cortical substrates of more

complex intelligence behavior. Secondly, the question of precisely what role motor cortex plays in these processes is a long-debated topic. [17] On the one hand, classical studies identifying the so-called motor areas of the cortex show that microstimulation of neurons in these areas produce movements in different parts of the body [11], and electrophysiological recordings show that motor cortex neurons are tuned to movement direction. These studies seem to imply that the motor cortex is a brain region tasked with generating and controlling movements. On the other hand, however, complete motor cortex lesions leave general motor abilities perfectly intact in many animals [7, 16, 17], suggesting that the biological substrates of motor control and generation may in fact lie in subcortical structures. [16]

At the very least it seems that, by assuming motor cortex is for generating movements, we may be missing the exact nature of what it does. One recent finding in this vein is that motor cortical lesions lead to anomalous behavior in response to unexpected changes in the environment. Lopes *et al* (forthcoming) trained rats to cross an obstacle course with eight rectangular steps. After several training sessions, some of the steps were loosened such that they were free to rotate when stepped on. On first encounter with steps in this unstable state, rats with motor cortical lesions responded drastically different from controls, immediately freezing in the middle of the crossing. Surprisingly, the lesioned rats performed exactly as the controls in all previous and subsequent trials with stable and/or unstable steps - it was only when a surprising event was introduced into the assay that rats with abnormal motor cortex were impaired.

This observation puts forth the hypothesis that motor cortex plays a crucial role in the formation of novel motor responses to unfamiliar situations. Indeed, one might claim that this is its primary function. The present study is an attempt to corroborate and extend this hypothesis by looking for an electrophysiological correlate of the motor cortical computations being carried out in predictable and unpredictable environments. We measured cortical responses by performing electrocorticographic (ECoG) recording of motor cortex in rats while they performed the same behavioral assay of Lopes *et al*, allowing us to obtain electrophysiological recordings of motor cortex during motor tasks in an environment with graded levels of uncertainty.

2. Methods

2.1. **Behavioral Assay.** Behavioral assay was exactly as in Lopes *et al* (forthcoming). During each experimental session, rats were put into a box that consisted of two reward ports separated by a corridor with 8 elevated steps. The rats had to cross this corridor to retrieve water rewards from each port. To do so efficiently, they had to learn to step on each of the steps, rather than in between them. This was learned quickly after the first few sessions, evident by an increase in crossing

speed and shift of posture after the first 3-4 sessions (fig. 1), replicating the original work. [17] To ensure rats were motivated to cross the obstacle course, they were water-deprived for 20 hours prior to each session. All sessions were performed in the dark, with infrared illumination for video recording.

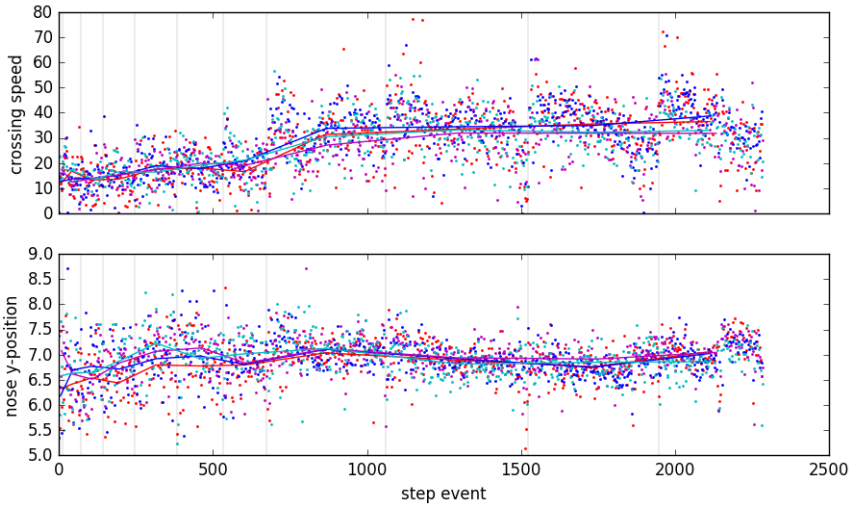

FIGURE 1. Crossing speed and vertical nose position at the moment of each stepping event analyzed in the *constant stability* sessions. Light gray lines demarcate boundaries between sessions, and solid colored lines designate session means for each stepping event: red and magenta correspond to the 3rd step encountered in left-to-right and right-to-left crossings, respectively, and blue and cyan similarly correspond to the 4th step encountered. Step events are arranged chronologically. Note the improvement in crossing ability across time: the rat gets faster and faster and progressively shifts its center of gravity up, as well as forward (horizontal nose position not shown), replicating previous results. [17]

Critically, each step's stability could be manipulated such that, when in the unstable state, it would rotate freely upon being stepped on. The stability of the center two steps was manipulated in this way according to three different conditions. In the *constant stability* condition (the first 11 sessions), the steps were always stable throughout the whole session. In the *rare instability* sessions (the next 7 sessions), the two middle steps were made unstable every 20 crossings. Unfortunately, due to logistical factors there was a 19-day break between the 5th and 6th of these sessions for the rat analyzed below. The last 3 sessions were assigned the *frequent instability* condition, where the middle two steps were made loose on random crosses, such that they would often be loose on back-to-back crossings. On all trials it was ensured that the rat could not know the state of the steps as in Lopes *et al.*

2.2. **Video Analysis.** The exact frame and time of each step event was extracted from the video by region of interest (ROI) analysis. For each step, an ROI was defined over its surface. Whenever a rat steps on a step, the mean pixel intensity over the step's ROI increases because the white rat paws are brighter than the step. By extracting frames where the mean pixel intensity was above a certain threshold (after performing background subtraction), we were able to obtain the frame times and images for the exact moments when the rat's forepaw made contact with each step in the assay. These are termed "step events".

To then identify what paw was used on each step event, we used an ROI defined just above the step being stepped on that contained the paw and part of the leg. Because the assay is only illuminated from the front, in a given frame the paw opposite from the light source with respect to the rat's body consistently appears darker than the other (i.e. left paw appears darker than the right paw on left-to-right crossings, and vice versa). We were able to exploit this to identify the stepping paw by using k-means clustering (k=2) on the ROI intensity histograms for all the trials within a session, doing this separately for each crossing direction.

2.3. **Electrophysiological Recordings.** To measure cortical responses, ECoG was used because of its improved signal-to-noise ratio relative to other extracellular recording methods such as electroencephalography. Because the electrodes are placed inside the skull, the attenuating and low-pass spatial filtering properties of the skull and scalp layers are avoided, allowing us to not only measure field potentials but also the so-called multi-unit activity enveloped (MUAe). [9]. Furthermore, ECoG has improved spatial resolution and eliminates contamination from electromyographic (EMG) signals. [4, 9] In the rat analyzed, two grids of 64 electrodes were placed over the motor cortex in the right hemisphere, one posterior to the other.

2.4. **Data Analysis.** Here, we analyze data from only one rat, using only stepping events corresponding to stepping on the 3rd and 4th steps encountered on any given crossing[1].

To reduce the dimensionality of the electrophysiological data, we often grouped the 128 total electrodes into 4 quadrants by averaging electrode responses in each quadrant (anterior-medial, anterior-lateral, posterior-medial, posterior-lateral). This approach was taken after observing that responses by nearby electrodes were very similar, with biggest distinctions across the posterior-anterior axis (i.e. across the separate electrode grids).

For all analyses below, the raw signal was pre-processed in one of two ways. Event-related field potentials (ERP) were obtained by band-pass filtering the raw

[1]Hereafter, 'step events' refers to these. The remaining four steps are not included in any analyses, and stepping events for the (3rd,4th) and (5th,6th) steps in the assay are only considered when they occurred in left-to-right and right-to-left crossings, respectively (i.e. when they were the 3rd and 4th steps encountered on that given crossing).

signal between 0.1 and 150 Hz (using a 3rd order butterworth filter, two-pass), and then averaging. We performed band-pass rather than low-pass filtering simply to remove the DC component (0 Hz) of the signal to facilitate comparison between different ERPs. The multi-unit activity envelope (MUAe) signal [9] was obtained by high pass filtering the raw signal with cutoff at 400 Hz (using 8th order butterworth filter, two-pass), and rectifying. Rectification was always performed after averaging the high pass-filtered signal. What exactly these two signals reflect is a long-debated topic, but it is generally thought that the ERP reflects synchronized neuronal inputs (i.e. post-synaptic potentials) [4, 5], whereas the MUAe signal is sensitive to individual neuronal outputs (i.e. action potentials), possibly reflecting the average spike rate of a population of neurons. [9] However, it is a caveat worth noting that the true physical sources of ECoG signals are not completely understood.

3. Results

3.1. **Step-evoked response.** We first attempted to identify an ECoG signal correlate of regular stepping on a stable step. Averaging over all step events across all *constant stability* sessions after the first one (which we excluded due to possible habituation effects, evident from the relatively small number of crossings in that session), we obtained a clear ERP for stepping on a stable step whilst in a predictable environment, shown in figure 2.

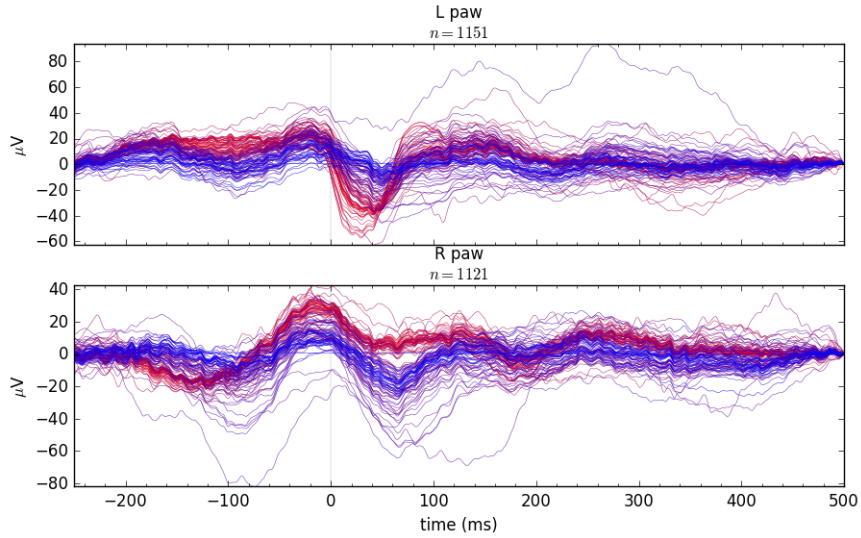

FIGURE 2. ERP for step events in the *constant stability* sessions. As mentioned above, this signal is band-passed between 0.1 and 150Hz (see Data Analysis section). Colors are arranged along red-blue spectrum by position along the anterior-medial to posterior-lateral direction: more red lines correspond to more posterior and more medial electrodes. Note that, for step events with the contralateral paw, the peak latency of the signal from these electrodes is quite short (30-40ms), progressively increasing for more and more posterior electrodes (up to 50ms). Also note the generally longer peak latency in the ERPs for ipsilateral step events (around 60ms).

The ERP consists of a systematic fall in potential initiating at about time of contact. The drop is steeper for contralateral paw step events than for ipsilateral step, peaking at about 30-40ms as opposed to 60ms. Due to the limitations of extracellular electrophysiological methods, it is hard to know the source of these dynamics or when exactly the dip initiates. [4] However, the 20ms difference in peak latency is consistent with previous voltage-sensitive dye (VSD) imaging of rat somatosensory cortex response to forepaw stimulation, where it has been shown that contralateral responses have greater amplitude and lower peak latency than ipsilateral responses. [20] This suggests the signal observed corresponds to a somatosensory response to step contact, albeit in motor cortex. Contrary to their findings, however, we found that the contralateral response seemed to originate in the medial-anterior areas of motor cortex and then migrated towards more lateral and posterior areas, whereas they found the spatial dynamics of the VSD signal in somatosensory cortex to follow a lateral to medial direction. [20]

3.2. **Unexpected environmental perturbations.** Having observed an evoked cortical response to stepping on a familiar stable step, we can now ask what the

cortical response looks like to an unexpected unstable step by shifting our focus to the *rare instability* condition. Here, blocks of 20 successive crosses with stable steps were succeeded by one crossing in which the two middle steps were made unstable. In these unstable trials, the presence of the instability in the obstacle course was certainly unexpected by the rat, at least the first time it occurred. These kinds of situations are exactly the kind that we might expect motor cortex to come into play under our hypothesis. Thus, we should expect a characteristic evoked response from unstable step events in this condition.

To examine this, we averaged the signal time-locked to step events corresponding to the fourth step encountered when it was unstable, averaging over all sessions in the *rare instability* condition. The resulting ERP is depicted in figure 3, and consists of of a characteristic biphasic component about 30ms after contact. This component is not at all present in the ERP for step events corresponding to stepping on an expected stable step. Could this signal reflect the motor cortical computations we have hypothesized are so important for dealing with unexpected events?

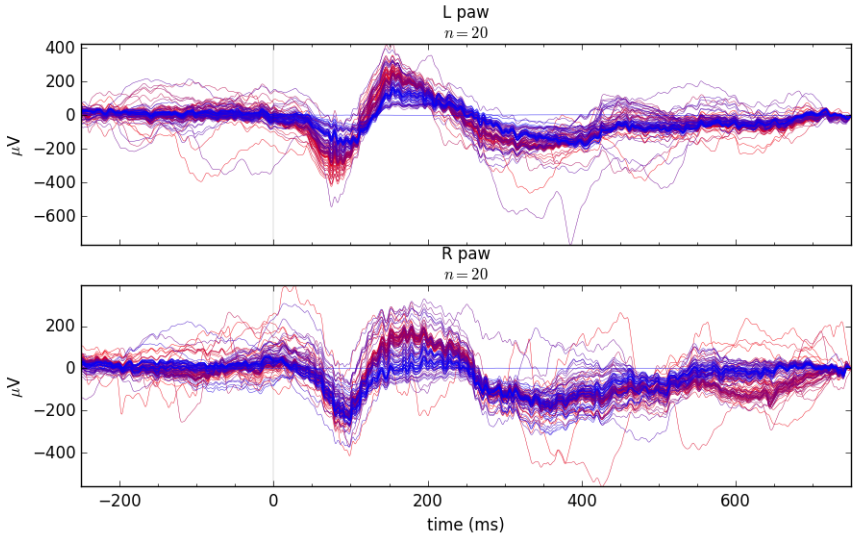

FIGURE 3. ERP for step events corresponding to stepping on an unstable step in *rare instability* sessions. Colors as in fig. 2

Three possibilities arise here. Firstly, the ERP could be reflecting the differential somatosensory stimulation resulting from the unstable step rotating under the weight of the paw upon stepping. This is certainly a possibility, but seems unlikely since the specific stimulation is likely to be very different from one trial to the next, depending on the exact position and crossing speed of the rat upon stepping, which was quite variable across all the trials considered (fig. 4). In fact, as discussed further below, the rat exhibited different behaviors upon encountering the stable

step. Whereas several times it was taken completely by surprise and slipped on the freely rotating step, other times it was cautious and immediately stopped itself before putting all its weight on the step, proceeding to then jump over it or stop to explore. Such large variability in the resulting somatosensory stimulation would be unlikely to result in such a clean systematic signal after averaging over only $n = 20$ trials. Moreover, the difference in peak latency between ipsilateral and contralateral responses is on the order of 5ms, which is significantly smaller than that found in the aforementioned VSD studies on somatosensory cortex responses to somatosensory stimulation. [20]

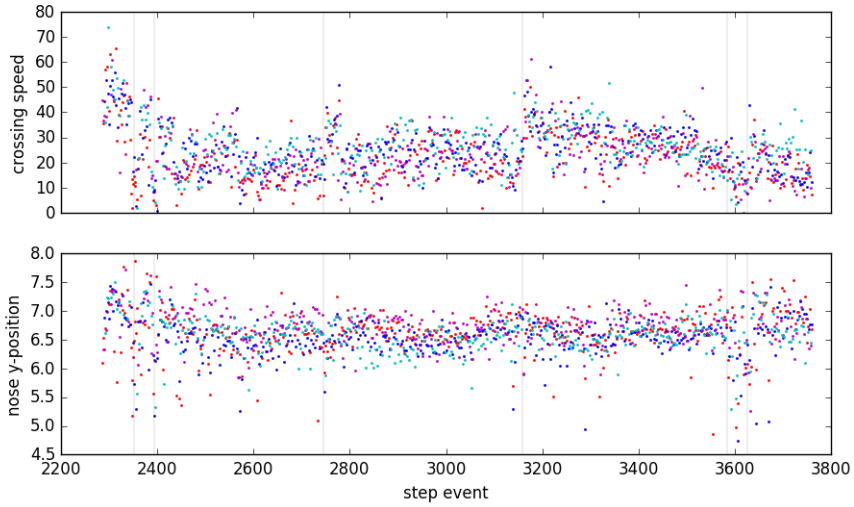

FIGURE 4. Same as fig. 1 but for *rare instability* sessions.

A second possibility is that this ERP reflects processes underlying the generation of the appropriate motor response to the loose step. The relatively small difference in peak latency between the contralateral and ipsilateral signals weighs against this, but we decided to explore this possibility further by examining the rat's behavioral response in each of the individual trials. We found that the rat generally exhibited one of two responses: exploring and jumping. Surprisingly, the rat studied here exhibited a "halting" response in its first encounter with an unstable step, whereby it simply froze, seemingly confused and unable to decide what to do next for about a second before finishing the crossing and exiting the corridor. This is the response typically exhibited by rats with motor cortical lesions. [17] In subsequent encounters with the unstable step, however, the rat analyzed here typically immediately stopped in its tracks and began exploring the unstable step and the ones next to it by sniffing and whisking. After about 10 such encounters, it began consistently jumping over the middle steps by using its hind paws to thrust it over the loose step after front paw contact.

If the ERP we found relates to movement generation, we would expect the signals to differ between these two fundamentally different motor responses. Averaging the signal over step events corresponding to each response[2], we found some slight differences in the ERP, but the biphasic component remains across both motor responses (fig. 5) with slightly larger amplitude in "jumping" response trials.

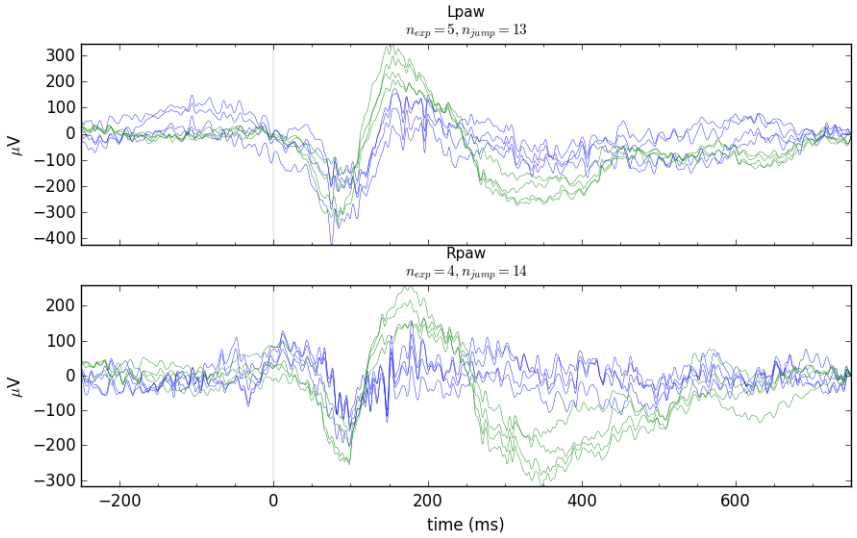

FIGURE 5. ERP for step events corresponding to stepping on an unstable step in *rare instability* sessions, separated by the subsequent behavioral response: exploring (blue) or jumping (green).

These findings seem to exclude the possibility that the biphasic component of the ERP reflects somatosensory or motor processing. Instead, it seems to be the cortical response to a violation of expectations. If we further look at the averaged MUAe signal for these trials, we find a significant increase relative to the MUAe present in stable stepping events (fig. 6). A possible hypothesis that falls out of these observations is that the motor cortex contains populations of neurons encoding the error between the rat's expectations about environment and what it actually observes, reminiscent of predictive coding theories of cortical function. [21, 10] This idea is also consistent with previous findings using a similar behavioral assay with cats. [19]

[2]Step events in which the rat produced idiosyncratic responses that did not fall into the "exploring" or "jumping" categories (such as the "halting" response observed in the first unstable step encounter) were omitted from this analysis. There were 4 such events, leaving 18 events for each paw.

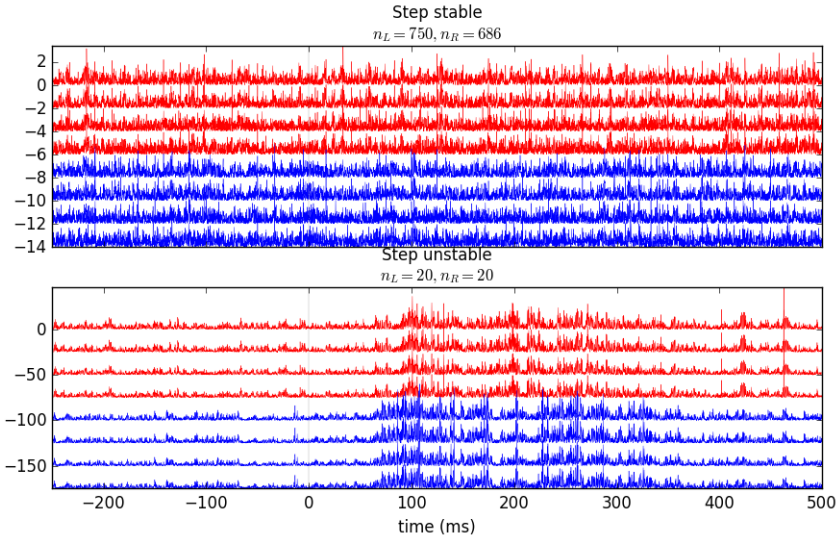

FIGURE 6. MUAe signal for step events corresponding to stepping on a stable step in the *constant stability* sessions (top panel) and to stepping on an unstable step in the *rare instability* sessions. Signals from all 128 electrodes averaged into four quadrants, arranged here, from top to bottom: anterior-medial, anterior-lateral, posterior-medial, posterior-lateral. The MUAe traces for each step event were plotted on separate axes because of the significantly smaller amplitudes for the stable step event MUAe, which likely reflect the larger sample size over which the signal was averaged. However, the difference between pre and post-step event amplitude that is apparent only in the unstable step event MUAe signal cannot be accounted by this. Red = left paw, blue = right paw

3.3. **Predictably unpredictable perturbations.** If this biphasic "surprise" ERP component indeed reflects the activity of populations of neurons encoding a violation of expectations, it should disappear when the presence of an unstable step becomes expected. Presumably, this is the case in the *frequent instability* sessions, where unstable steps are interspersed randomly throughout crosses. Indeed, by averaging over unstable step stepping events in these sessions, we find that the biphasic component of the ERP disappears, and the increase in MUAe amplitude after an unstable step event becomes significantly smaller (5 μV as opposed to 20μV, fig. 7).

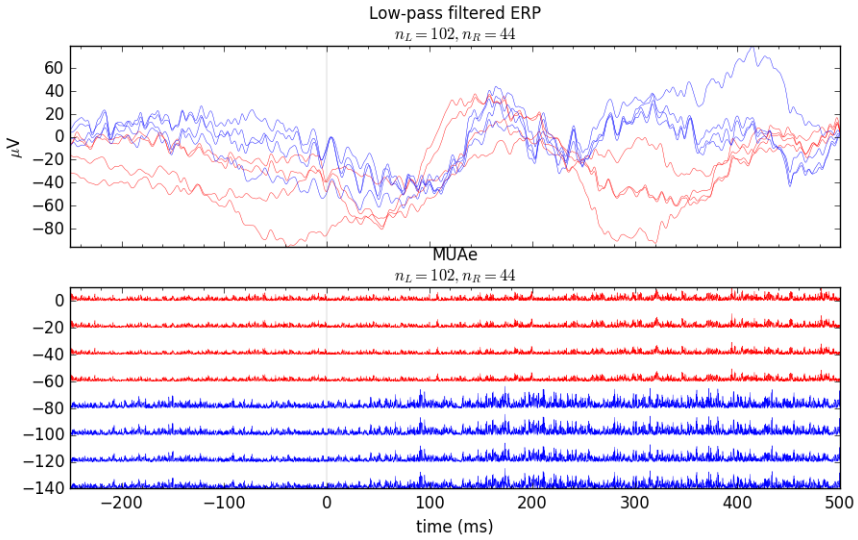

FIGURE 7. ERP and MUAe signal for step events corresponding to stepping on an unstable step in *frequent instability* sessions. Red = left paw, blue = right paw.

Importantly, if we look at individual unstable step trials within this condition, it can be seen that our purported "surprise" ERP component is in fact present in the signal for early *frequent instability* unstable step events, before quickly disappearing (fig. 8). This is easily accounted for by our hypothesis in that unstable steps are unexpected at first, as in the *rare instability* condition. However, once the rat encounters sufficient unstable step trials, it begins to expect them and the "surprise" is no longer there.

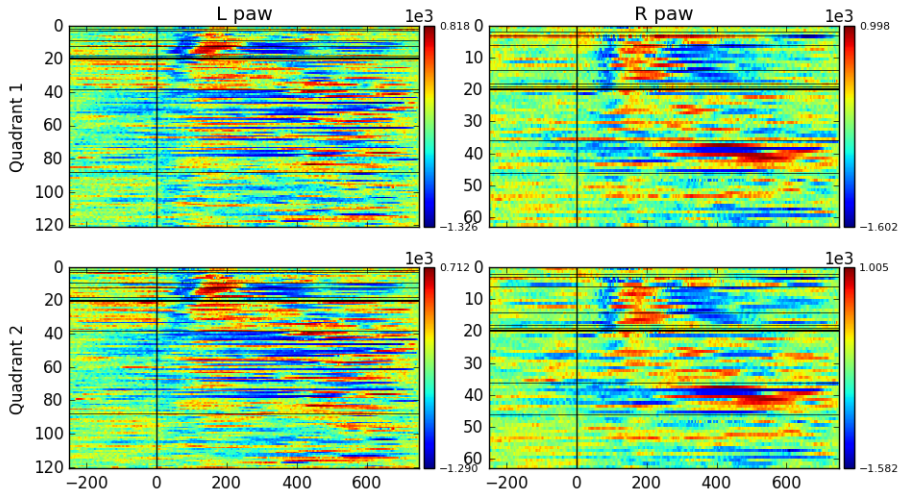

FIGURE 8. ERP image with individual band-pass filtered signals for all crosses in the *rare instability* and *frequent instability* sessions. We focused here only on the anterior electrode quadrants, where the "surprise" component was most present. Black lines denote boundaries between sessions, and the thick one indicates change from *rare instability* to *frequent instability* conditions. Note the red blobs just after step event that are present above and just below this thick black line, reflecting the presence of the "surprise" ERP component in *rare instability* trials and early *frequent instability* trials, but not late *frequennt instability* trials.

If we further divide the *frequent stability* trials by the stability of the step in the immediately preceding five trials, this explanation is further corroborated: the unstable step event ERP contains the biphasic component only when the step was stable in the preceding five trials (fig. 9, top panel). This is easily accounted for by the idea that the biphasic component reflects violation of expectations, as an unstable step should be more unexpected when it was stable in the five immediately preceding encounters. It is worth noting, however, that in this case the peak latencies are much greater than in the original biphasic response found in the unstable step ERP in the *rare instability* trials (fig. 3). Furthermore, we should expect that the opposite will hold - that the biphasic component should appear in the ERP for stable step events when the step was unstable in the preceding 5 trials. This was not found (fig. 9, bottom panel). Part of the discrepancies (or the effects!) here may lie in the relatively small sample sizes, so more work needs to be done to confirm or disprove the idea of a "surprise" ERP component.

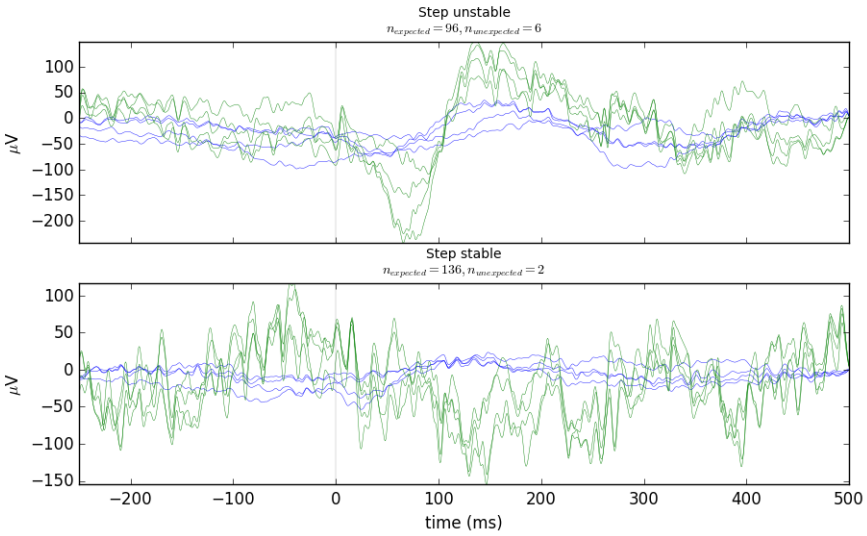

FIGURE 9. ERP for step events corresponding to stepping on the 4th step encountered when it was unstable (top) or stable (bottom) in the *frequent instability* sessions. Only left paw step events were analyzed, because the rat rarely used right paw on 4th step encountered in these sessions. Green = trials where instability/stability was unexpected (i.e. the step was was stable/unstable in the five immediately preceding trials), blue = trials where instability/stability was expected (the step was instable/stable in at least one of the preceding five trials

4. DISCUSSION

Here, we identified a characteristic electrocorticographic response in rats that seems to reflect motor cortical processing arising from the violation of expectations. What computations are exactly being performed by the neural activity giving rise to this response is an open question. One interesting possibility is that this response arises from neuron populations encoding prediction error [21, **?**]. This would account for the elevated MUAe amplitude observed immediately succeeding the unexpected event: when the prediction error is large (i.e. when expectations are violated), these neurons fire at a higher rate. [9] Another possibility is that the motor cortex is computing and planning a tailored motor response to the novel environment. [16]

To flesh out the implications and details of the electrocorticographic response observed here, it will be necessary to employ more sophisticated signal processing techniques [6], that were not used here due to time constraints. Primarily, it will be elucidating to do time-frequency power decomposition of the several ERPs discussed here: might neural activity reflecting the violation of expectations be

constrained to a certain frequency band? Other interesting possibilities are independent components analysis to identify different components of the ERP trace for each step event [18], and microstate analysis. [1]

The obvious further step after this towards understanding exactly what kinds of computations the motor cortex is to obtain more detailed information about the underlying neural activity. As mentioned above, the precise physical sources of electrocorticography measurements are not known, so our signal may be contaminated by subcortical activity. To precisely measure cortical neuron activity will require higher spatial resolution and higher signal-to-noise ratio recording techniques, such as single cell recording methods. As shown here, combining neural recordings with individual trial behavioral analysis can yield powerful insights.

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