Mesoscale cortex-wide neural dynamics predict self-initiated actions in mice several seconds prior to movement

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ABSTRACT

Volition - the sense of control or agency over one's voluntary actions - is widely recognized as the basis of both human subjective experience and natural behavior in non-human animals. To date, several human studies have found peaks in neural activity preceding voluntary actions, e.g. the readiness potential (RP), and some have shown upcoming actions could be decoded even before awareness. While these findings may pose a challenge to traditional accounts of human volition, some have proposed that random processes underlie and explain pre-movement neural activity. Here we seek to address part of this controversy by evaluating whether pre-movement neural activity in mice contains structure beyond that present in random neural activity. Implementing a self-initiated water-rewarded lever pull paradigm in mice while recording widefield [Ca++] neural activity we find that cortical activity changes in variance seconds prior to movement and that upcoming lever pulls or spontaneous body movements could be predicted between 1 second to more than 10 seconds prior to movement, similar to but even earlier than in human studies. We show that mice, like humans, are biased towards initiation of voluntary actions during specific phases of neural activity oscillations but that the pre-movement neural code in mice changes over time and is widely distributed as behavior prediction improved when using all vs single cortical areas. These findings support the presence of structured multi-second neural dynamics preceding voluntary action beyond that expected from random processes. Our results also suggest that neural mechanisms underlying self-initiated voluntary action could be preserved between mice and humans.

INTRODUCTION

Over the past several decades studies of volitional, i.e. free and voluntary, action in humans using self-initiated (i.e. uncued) behaviors such as flexing a finger or pressing a button have shown that prior to movement there is a gradual increase in scalp electroencephalography (EEG) signal over pre- and supplementary-motor-area (pre-SMA and SMA, respectively; Ball et al., 1999; Cunnington et al 2002). This increase in activity is known as the "readiness potential" (RP; Kornhuber & Deecke, 1964, 1965; Deecke, Grözinger, & Kornhuber, 1976; Deecke & Kornhuber, 1978; Libet 1983; Shibasaki and Hallett 2006) and has received increasing attention with some interpretations that it is evidence that voluntary decisions might be made prior to awareness with several studies replicating and extending the original work (Haggard & Eimer, 1999; Schlegel et al., 2013; Sirigu et al., 2004; Alexander et al. 2016). Additionally, single neuron physiology studies have also shown a significant increase (or decrease) in the firing rate of single neurons in SMA and pre-SMA (as well as anterior cingulate cortex; ACC) prior to movement (Fried et al 2011). In parallel, human functional magnetic resonance imaging (fMRI) studies have shown that upcoming behaviors could be decoded up to several seconds prior to movement (Soon et al 2008; 2013; Bode et al 2011; Colas and Hsieh 2014). The role of pre-movement neural activity in voluntary behavior is the subject of active debates on human decision making including free will (Jahanshahi and Hallett, 2003; Lang, 2003; Shibasaki and Hallett, 2006; Haggard, 2008; Klemm, 2010; Custers and Aarts, 2010; Schurger 2012; Deecke 2012; Guggisberg and Mottaz, 2013; Bode et al., 2014; Maoz et al 2015; Lavazza 2016; Schurger et al 2016). These debates on the neural genesis of voluntary action are further complicated as other studies have shown volitional actions are more likely to occur during certain phases of breathing (e.g. exhalation; Park et al 2020) or phases in cumulative neural activity (ie. the crest in slow cortical potential - SCP; Schmidt et al 2016) which have no immediately obvious connections to volitional intent, awareness, neural noise, or external stimuli or cues.

Although some have called for more ethologically relevant self-initiated behavior studies (e.g. Mudrik et al 2020), it remains challenging to implement them in humans especially in cue-free paradigms. First, neuroanatomically precise high-temporal and spatial precision recordings from many areas and rare in humans (though some limited studies exist, e.g. Fried et al 2011). Second, obtaining statistically sufficient numbers of trials (i.e. tracking behaviors for days or weeks; see Bode et al 2014 for a discussion) of higher-value salient actions (e.g. important decisions that are made naturally outside of laboratory environments) is not yet possible in humans. Additionally, human laboratory protocols for volitional studies (e.g., the subject being told to act freely in a study) may result in instructed - rather than free behavior - and there are concerns about whether human subjects can act randomly (Lages et al 2013) or otherwise carry out balanced behaviors (e.g. randomly pressing left vs. right button) in voluntary behavior paradigms (Bode et al 2014). An alternative approach to studying self-initiated action is to characterize the neural correlates of voluntary action and removing the requirement for reporting intent or awareness altogether (similar to some human studies, e.g. Soon et al 2008, 2011; Bode et al 2011). This avoids some of the challenges of human paradigms and makes it possible to implement non-human animal models where more ethological valuable actions could be available (i.e. food or water seeking behaviors) and higher resolution intracranial neural recordings can be made during hundreds or thousands of trials. There is evidence to support this direction as several non-human animal studies have identified structure - or increases - in pre-movement neural activity in non-human primates (Romo and Schultz 1986; 1990; Coe et al., 2002; Lee and Assad 2003; Maimon and Assad 2006; Ding and Hikosaka, 2006), rodents (Hyland 1998; Isomura et al 2013; Murakami et al 2014), crayfish (Kagaya and Takahata, 2010), zebrafish (Lin et al 2020). However, none of these studies were designed to - nor report - the neural structure of voluntary, or self-initiated actions, and they do not evaluate the predictive relationship between pre-movement cortical neural activity and voluntary self-initiated behaviors.

Here we report results obtained from a voluntary behavior paradigm targeting the decoding of future body movement and rewarded actions from neural activity in mice. Using a self-initiated voluntary task and widefield [Ca++] cortical imaging (Silasi et al 2016; Vanni and Murphy 2014) we tracked both water rewarded lever pull behavior of water deprived mice as well as spontaneous body movements. We gathered hundreds to thousands of self-initiated actions over months of recordings and collected neural activity from several cortical areas. We find that both self-initiated water rewarded lever pulls and spontaneous body movements could be decoded above chance a few to several seconds prior to movement initiation from neural activity. We show the voluntary movement neural code is distributed across multiple cortical areas and additionally replicate and extend several findings from human studies. Our study is in line with accounts of pre-movement neural activity having temporal and spatial structure beyond that present in random neural dynamics and supports a causal role between pre-movement neural activity and action that is on the scale of several seconds prior to action.

RESULTS

Fig 1: Tracking and decoding self-initiated behaviors from widefield neural activity in mice

Figure 1 here

Several studies of voluntary actions, such as finger or wrist movements, in humans have identified an increase in scalp EEG signal over SMA and pre-SMA - known as the RP - occurring 0.5sec to 1.5sec prior to movement and in some cases awareness of movement (Fig 1a, b; Kornhubber and Deecke 1964, 1965; Libet et al 1983; Ball et al., 1999; Cunnington et al 2002). We developed an analogous self-initiated voluntary behavior paradigm in mice to characterize pre-movement neural activity while recording widefield [Ca++] activity from cortex (Fig 1c, d, Sup Figs 1.1, 1.2; see also Methods). Mice were headfixed and trained to perform a self-initiated lever-pull to receive a water reward without sensory cues or stimuli (see Methods).

Self-initiated movements in mice are preceded by neural activity increases several seconds prior to movement. Similar to the RP in humans, voluntary behaviors in mice are preceded by an increasingly stereotyped average widefield [Ca++] signal up to 10 seconds or earlier in several areas including motor and limb cortex (Fig 1e, Sup Figs 1.3, 1.4). This common dynamical pattern was observed in all sessions and animals but not when considering random segments of neural activity (Sup Fig 1.3,1.4).

Self-initiated movements in mice can be decoded seconds prior to action from preceding neural activity. In addition to the human RP, several human fMRI studies have shown that voluntary behavior could also be decoded up to several seconds prior to movement, usually a few percent above chance (Fig 1f,g; Soon et al 2008; 2013; Bode et al 2011). To compare with human studies, we trained support-vector-machines (SVMs) using trials within each session to decode upcoming rewarded lever pulls (Fig 1h) or spontaneous limb movements (Fig 1i,j). We decoded, i.e. classified, (i) neural activity preceding a voluntary action (e.g. rewarded lever pull) vs (ii) neural activity representing random periods of behavior similar to two class voluntary choice decoding carried out in humans (e.g. Soon et al

2008; note: we defined random activity as continuous segments of neural data that were centered at least 3 sec outside of lever-pull times; see Methods). Within each session we used sliding windows of 1sec of neural activity ranging from -20sec to 0sec as input to the SVMs. Additionally, we trained markerless pose estimation methods to track the spontaneous limb movements of mice (see Methods). Upcoming rewarded lever pulls could be decoded several seconds prior to movement with decoding accuracy curves improving closer to the lever pull time (examples in Fig 1h). Similarly, spontaneous limb movements were also decodable above chance a few seconds prior to movement (examples in Fig 1i,j).

In sum, neural activity preceding self-initiated lever pulls in mice is preceded by multi second stereotyped increases in widefield [Ca++] cortical activity similar to the human RP. Similar to human fMRI results, upcoming lever pulls or spontaneous limb movements could be decoded from preceding neural activity in many cases with decoding accuracy greater than the previously reported in humans (e.g. Soon et al 2008; Bode et al 2011). Our findings suggests that, like humans, mice may also engage pre-movement neural dynamics spanning several seconds prior to voluntary action.

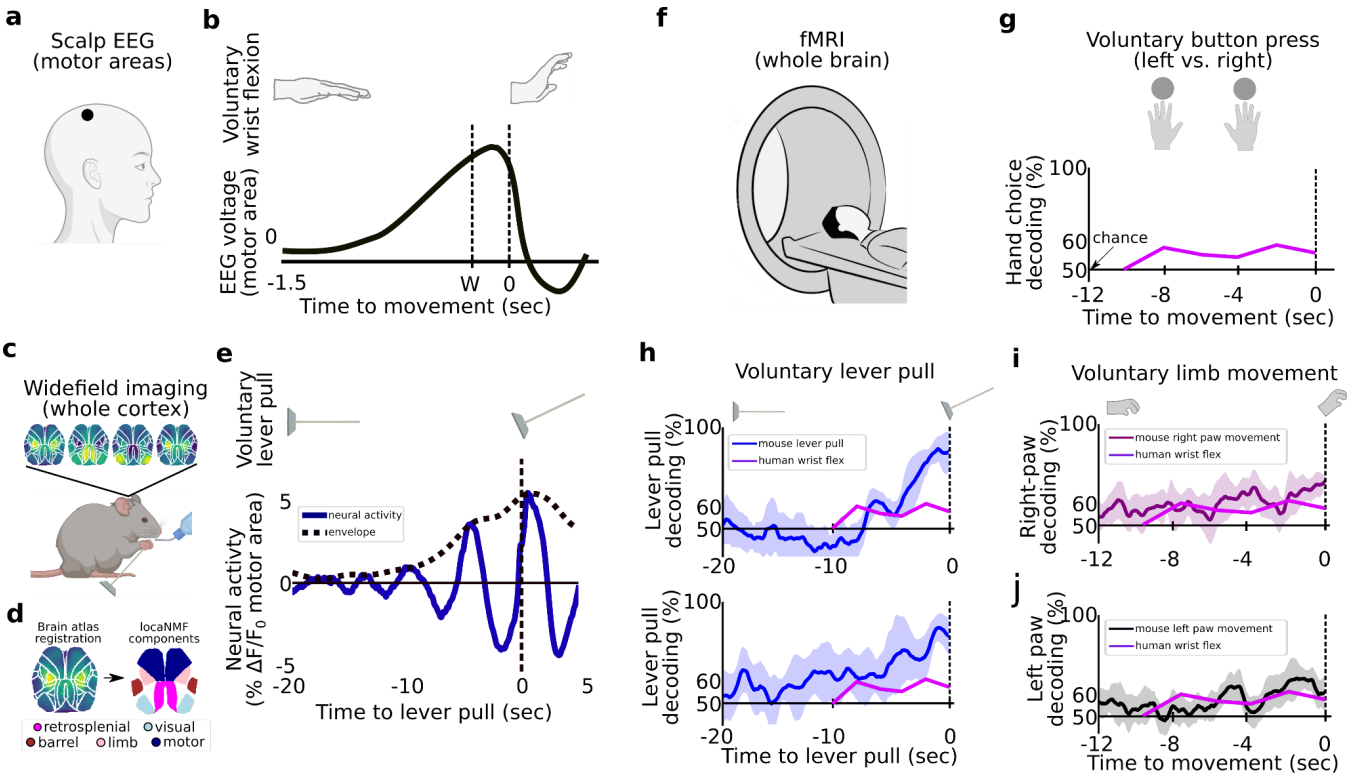


Figure 1. Detecting and decoding upcoming self-initiated mouse behaviors via widefield calcium activity. (a) Human voluntary behavior studies using scalp EEG target motor cortex and related areas. **(b)** Human voluntary wrist flexion studies recording EEG from motor areas (as in (a)) reveal an increase in neural activity 0.5-1.5sec prior to behavior initiation (t=0sec) or even awareness (t=W) in some studies. **(c)** Mice learn to voluntarily self-initiate a lever-pull to receive water reward while widefield [Ca++] activity is captured at 30Hz. **(d)** Allen Brain Atlas and locaNMF decomposition of neural activity into neuroanatomical areas (see also Methods). **(e)** The average motor cortex widefield calcium (neural) activity (solid blue line) becomes increasingly stereotyped prior to voluntary lever pull (t=0sec; dashed line represents Hilbert transform of oscillatory signal). **(f)** Human studies seeking to decode voluntary choice relying on fMRI imaging during voluntary behaviors. **(g)** Decoding accuracy for left vs. right hand voluntary button presses (solid purple line) is a few percent above chance several seconds prior to movement initiation and peaks at <10% above chance at movement time. **(h)** Two examples of decoding accuracy for rewarded lever pull vs. random states in mice (solid blue lines; shading is the

standard deviation of 10 fold cross-validation accuracy) showing increases seconds prior to movement and peaking > 30% above chance at movement time. **(i)** Similar to (h) but for decoding spontaneous mouse right paw movements (top; solid purple line) or left paw movements (bottom) reveal increases in decoding accuracy several seconds prior to movement with peak accuracy >10% at movement time. Panels 1a,b based on and adapted from Libet et al 1983; Panel 1g based on and adapted from Soon et al 2008.

Fig 2. A cortex wide distributed multi-second neural code underlies self-initiated actions

Figure 2 Here

We sought to systematically evaluate decoding accuracy for upcoming behaviors in different animals, using different cortical anatomical areas and for different movements (e.g. water rewarded lever pulls or spontaneous limb movements; Fig 2).

Decoding future rewarded lever pulls seconds prior to movement. We next focused on decoding water rewarded lever pulls: i.e. lever pulls that reached a minimum lever angle and were not preceded by a previous lever pull for at least 3sec (Fig 2a; Sup Fig 2.1; see also Fig 4 and Methods). Going back in time from t=0sec, we defined the earliest-decoding-time (EDT) as the last point in time at which the SVM accuracy was statistically higher than chance (Fig 2b, 10 fold cross-validation pval < 0.05, student 1 sample t-test corrected for multiple hypotheses using the Benjamini-Hochberg method; see Methods). The decoding accuracy was better than chance seconds prior to movement and gradually increased closer to the lever-pull (i.e. t=0 sec). EDTs ranged from 0 sec (i.e. lever pull was not predicted) to more than 13 seconds in some sessions (see Fig 2c for example EDTs). We also found a correlation between the number of trials within a session and EDT suggesting that EDTs could in principle be even lower (i.e. earlier decoding in time) than reported in our study (Fig 2c linear fit; note each EDT was computed from a single session; see also Methods). To evaluate this correlation, trials from sequential sessions were concatenated to obtain at least 200 cumulative trials resulting in improvement in EDT (Figs 2d,e). Pooled sessions EDTs were lower for all mice (Fig 2f; pvals < 0.01 for all animals).

Preparation of upcoming lever pulls is widely distributed across the cortex. We next evaluated decoding of upcoming lever pulls using individual cortical areas rather than the entire dorsal cortex (Fig 2g). Anatomically-informed components were obtained using LocaNMF (Saxena et al 2020; see Methods) and EDTs were computed for bilateral activity from: retrosplenial, somatosensory-barrel, somatosensory-limb, visual and motor cortex (Fig 2g). Somatosensory-limb cortex was generally the

most informative of upcoming lever pulls (i.e. lowest mean EDTs across all mice) followed by motor cortex; visual cortex based decoding had the highest EDTs (i.e. closest to lever pull time t=0sec). More importantly, using all regions for decoding yielded lower EDTs than using somatosensory-limb cortex alone (2 sample KS tests $<1E^{-5}$ for all comparisons; Fig 2g). These findings are consistent with a single neuron study in humans that showed pooling neurons yielded increased decoding accuracy of upcoming voluntary action when compared to single neuron decoding alone (Fried et al 2011) but our results yield earlier decoding times than previously shown in humans.

EDTs of self-initiated licking and paw movements are similar to those for lever pulls. Most human studies on self-initiated voluntary behavior employ simple behaviors such as the flexing of a finger or pressing of a button with a specific hand (e.g. Libet 1983; Soon et al 2008). Accordingly, we also sought to determine whether mouse spontaneous paw movements (not just those related to lever pulls) could be decoded from preceding neural activity (Fig 2h; see Methods for description of body movement tracking methods). Briefly, we defined a self-initiated body movement as the time when the body part increased its velocity to more than 1 x the standard deviation of all movement within the session (we also implemented a 3 sec non-movement lockout period as in human studies and as in the preceding section). SVMs were trained as for rewarded lever pull times but using the body movement initiation time (i.e. t=0). As for self-initiated lever-pulls, a strong correlation was present between the number of trials within a session and the EDT suggesting that with higher number of body movements (e.g. longer sessions) EDTs could be even lower (Fig 2i). Across all animals, upcoming body movements could be predicted above chance a few seconds prior to movement in the vast majority of sessions and in some cases more than 10 seconds prior to movement (Fig 2j). Importantly, with a few exceptions, licking or paw movements EDT distributions were not statistically different from lever pull time ETDs.

In sum, upcoming self-initiated behaviors in mice can be decoded above chance several seconds prior to movement for all animals with a strong dependence of the EDT on the number of trials present in each session. Single anatomical area analysis revealed that somatosensory-limb cortex contained the most information about upcoming movements - but that decoding information was distributed across multiple regions of cortex. Lastly, spontaneous body movements were decodable on similar time scales as water rewarded lever pulls.

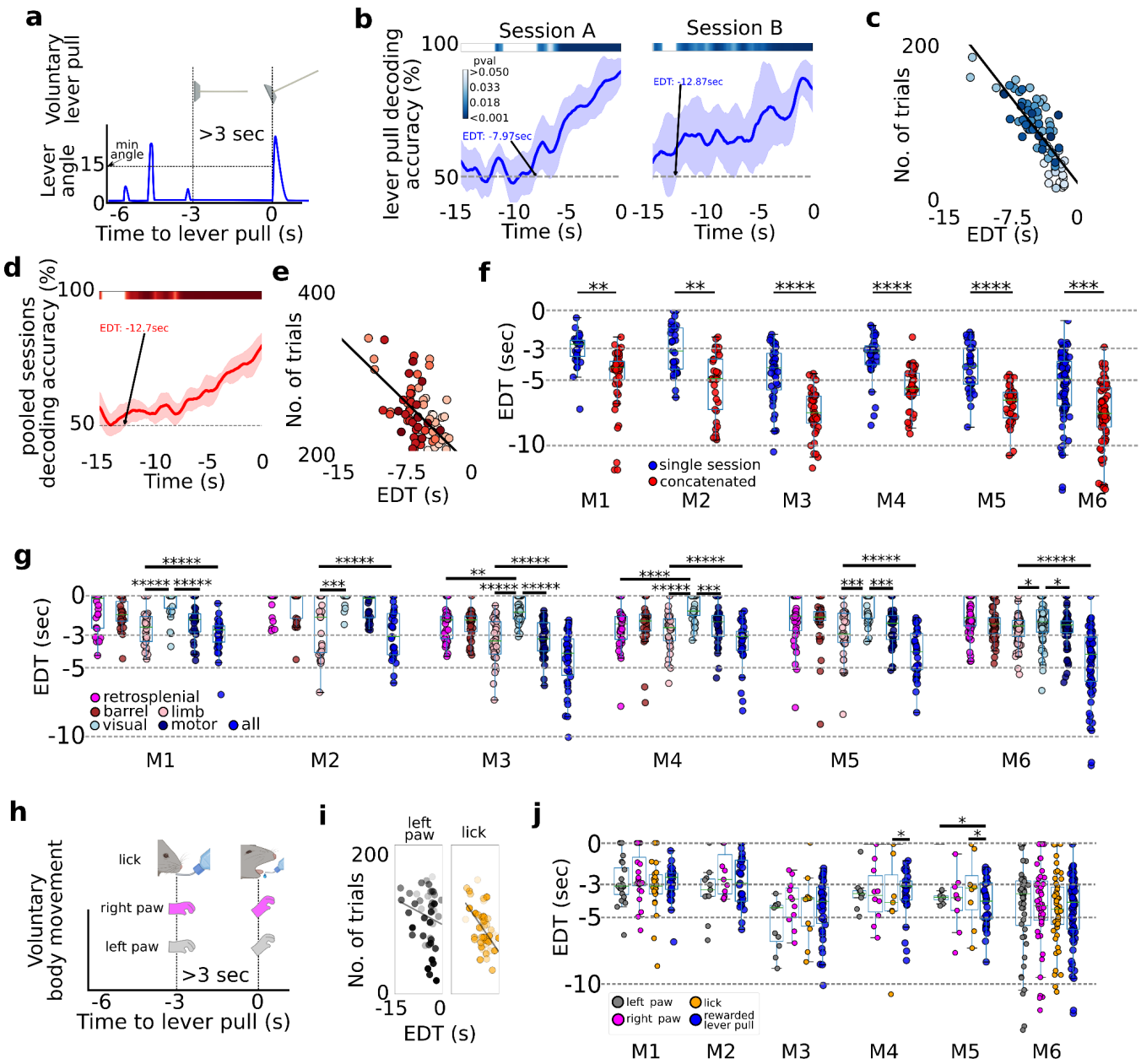


Fig 2. Decoding self initiated body movements using cortical neural activity. (a). Decoding self-initiated water rewarded lever pulls using a minimum 3 sec lockout window and a minimum lever angle threshold (see also Methods). **(b)** SVM decoding accuracy curves of two different sessions (mouse M4) reveal increased decoding accuracy near lever pull time and an earliest decoding time (EDT) of several seconds (curves represent average accuracy and shaded colored regions represent standard deviation over 10-fold cross validation; top colored bars represent p values of student t-test with a

Benjamini-Hockberg correction for multiple hypotheses; see Methods for more details; note sessions shown were atypical and were selected to illustrate decoding curves for very early EDTs). **(c)** EDTs from all sessions (mouse M6) show a strong correlation between EDT and the number of trials within a session (lighter colors indicate earlier sessions in the experiment). **(d)** Same as (b) but for an example from concatenated, i.e. multi-session, analysis. **(e)** EDTs for concatenated sessions (M6) also show a correlation between EDT and the number of trials present in the session and the earliest decoding time (lighter shading representing earlier sessions in training). **(f)** EDT distributions across all mice for single session trials (blue) vs. multi-session trials (red) reveals a significant improvement (i.e. a decrease in time) in EDTs for multi-sessions across all animals (cyan box plots show 25th percentile, median and 75th percentile). **(g)** Same as (f) but decoding using single anatomical areas reveals somatosensory-limb cortex is the most predictive of upcoming behaviors with visual cortex the least predictive. Decoding using all areas (blue dots) was more predictive than using somatosensory-limb cortex alone. **(h)** Decoding self-initiated licking, left and right paw movements using a minimum 3 sec lockout period. **(i)** decoding of left paw movements and licking (mouse M6) shows correlations with number of trials in the session. **(j)** EDT distributions for all sessions and mice were similar in most animals to decoding rewarded lever pulls (note mouse M2 licking behavior was not available; see Methods).

Fig 3. Self-initiated lever pulls occur during narrowly distributed slow-oscillation phases

Figure 3 Here

Work in human voluntary behavior has shown that slow-cortical potentials (SCPs), i.e. slowly changing voltages measured usually via EEG < 1Hz, might be involved in modulating voluntary behavior (Jo et al 2013; Schmidt et al 2016). In particular, voluntary behavior was found more likely to occur (on the order of ~10%) when the SCP phase over motor areas was near the crest. Additionally, there is some evidence to support that the SCP is related to awareness or consciousness and may play a causal role in internal state driven action (He and Raichle 2009; Northoff G. 2007). Given these findings we sought to determine whether self-initiated lever-pulls in mice co-occurred with specific phases of widefield [Ca++] activity (Fig 3).

Lever pulls occur during narrowly distributed phases of neural activity. As shown above (see Fig 1, Sup Fig 1.3,1.4) within single cortical areas, the neural dynamics preceding rewarded lever pulls become increasingly stereotyped closer to t=0sec (Fig 3a-top for an example of left forelimb neural activity). Fitting sinusoids to the last 5sec period prior to movement in each trial yielded sinusoidal fits with very similar phases at t=0sec (Fig 3a-bottom). Neither of these stereotyped dynamics were present in random segments on neural activity (Supplementary Fig 3.1). The phase distribution for a single session and cortical area was narrowly distributed with most phases falling in a < 90^0 wide window in many sessions (and < 45^0 for some sessions) (Fig 3b for example distribution from phases in Fig 3a; see also Sup Fig 3.2 for examples from all cortical areas). In contrast, random segments of neural activity had widely distributed phases (Fig 3c, Sup Fig 3.2). Computing the t=0sec phases for all trials across all sessions also revealed narrowly distributed phases (Fig 3d) in contrast to random segments of neural activity which yielded an approximately uniform distribution (Fig 3e). Similar human studies where the most likely SCP phase during voluntary action was the crest phase with ~30% probability followed by the rising phase (Fig 3f for an adapted example), phases in mice were also biased to these two locations

(i.e. crest or rising phase). In contrast to human results, we found an even higher bias as some mice had more than a 50% probability of initiating action in the crest phase - while others preferred the rising phase (See Fig 3g for 2 examples). The phases for all mice, sessions and trials showed that virtually all mice and cortical areas had narrowly distributed phases (Fig 3h) significantly different than random segments of neural activity (Fig 3i; Rayleigh test for uniformity $<<1E^{-5}$ for all animals and areas). The diversity of phase preferences was present not only between mice but also within mice as the phase bias could be significantly different between cortical areas (for example, Fig 3h, mouse M1 limb vs motor cortex phase differences). Lastly, the median inter-area phase correlation varied with some mice having highly correlated phases across areas whereas others having mostly low correlations (Fig 3j; see also Methods).

In sum, the phases of neural activity at lever pull initiation from all areas were significantly stereotyped, consistent with human findings (Jo et al 2013; Schmidt et al 2016). In contrast to human findings we found a higher bias of phases: i.e. behaviors were more likely to occur during a specific phase in mice than in humans. These findings support the presence of biases in neuroanatomical dynamics preceding self-initiated behavior preparation while confirming inter-animal differences in both anatomy and dynamics observed in other findings (see also Figs 4, 5).

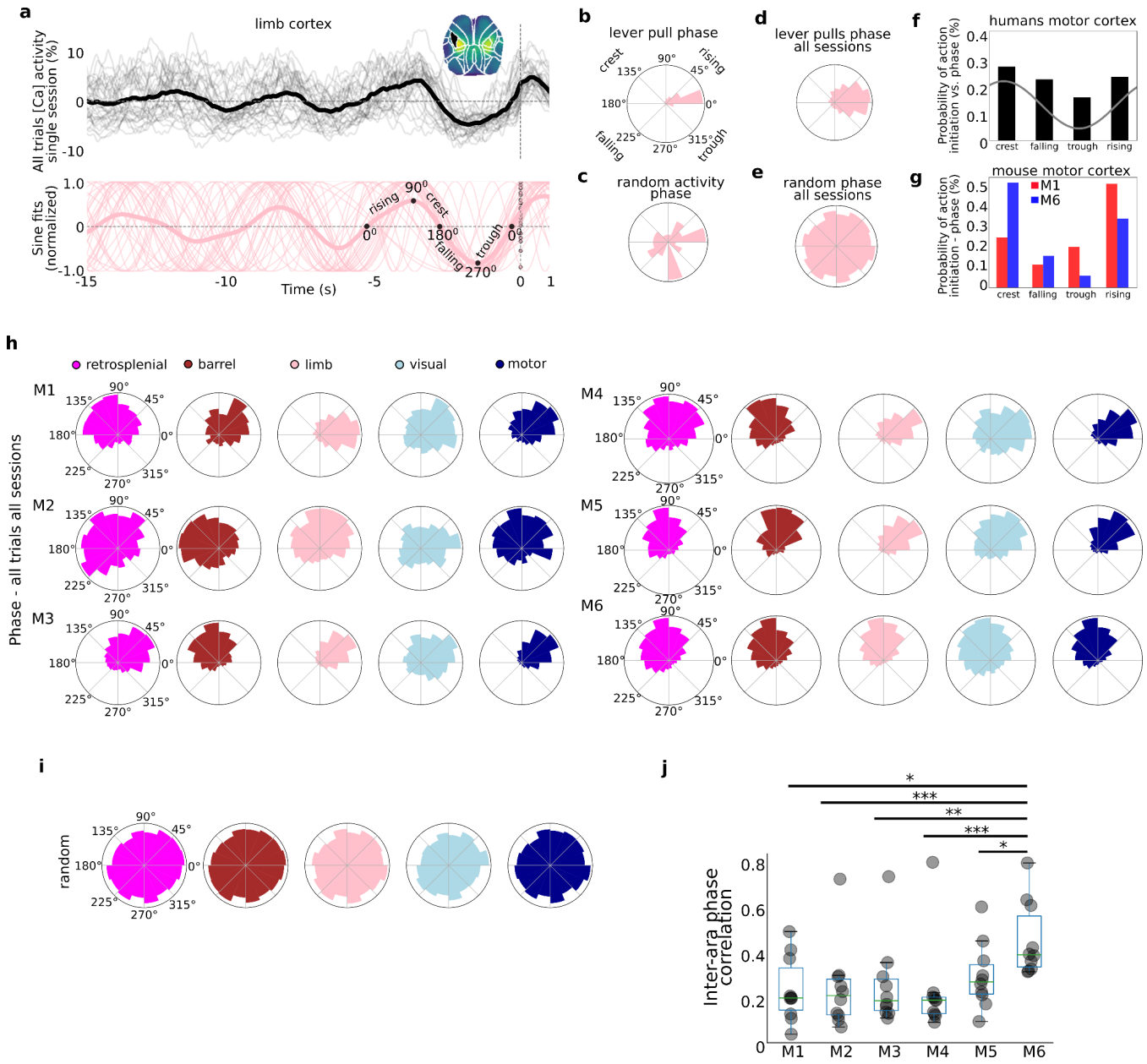


Fig 3. Self-initiated behaviors occur during specific phases of slow-oscillations. (a) Top: single trial neural activity (gray curves) from 33 trials in a single session (M4) for somatosensory-upper left limb cortex contain oscillations that become increasingly stereotyped closer to lever pull time (t = 0 sec; thick black curve is session average; inset shows anatomical area selected); Bottom: single trial sinusoidal fits (thin pink curves) to neural activity (in Top) and phases (scatter dots on the t=0 sec line; thick pink curve is session average). **(b)** Polar plot of results in (a) showing the distribution of sinusoidal fit phases at

t=0sec. **(c)** Same as (b) but for random periods of neural activity (i.e. not locked to any behavior). **(d)** Same as (b) but for all sessions in mouse M4. **(e)** Same as (d) but for random periods of neural activity across all sessions in mouse M4. **(f)** Probability of voluntary action in humans during various phases of the SCP. **(g)** Same as (f) but widefield [Ca++] from the motor cortex in mice M1 and M6. **(h)** Phase distributions across all mice, sessions and areas. **(i)** SCP phase distributions for random segments of neural activity for all areas in mouse M4. **(j)** Single trial pairwise correlation between all cortical areas (e.g. limb vs. motor, limb vs. retrosplenial etc.). Panel (f) adapted from Schmidt et a. 2016.

Fig 4. Decoding improves and neural dynamics become more stereotyped with weeks of increased task performance

Fig 4 here

We next sought to determine whether learning or mere longitudinal performance of our task changed the decoding accuracy or cortical neural dynamics during voluntary behavior initiations (Fig 4).

EDTs improve longitudinally in performing but not in non-performing mice. Considering the number of rewarded lever pulls per session, we found that between the first and last days of the experiment four of the mice (M2,M3,M5 and M6) increased the number of rewarded lever pulls while two of the mice (M1 and M4) decreased their number of pulls (Fig 4a; inset pearson correlation; M1,M2,M3,M4, M6 p values < 0.05; see Methods). We labeled the two groups as "performers" and "non-performers", respectively, to reflect the trend of performing increasingly more or less lever pulls in our task. SVM decoding accuracy curves over the weeks or months of behavior also revealed qualitatively different trends (Fig 4b for examples from 2 mice). Across all mice and sessions, performer mice exhibited a trend of decreased EDTs between the first and last days while non-performing mice had an increase in EDTs (Fig 4c; inset pearson correlation; M1,M2,M3,M6 p values < 0.05; M4 and M5 p values >0.05; see Methods).

Lever-pull neural activity space increases its cortical representation over time in most mice. We implemented a convex-hull based analysis to capture how the neural activity "space" prior to lever pulls changed over weeks or months of performance (Figs 4d-g; see Methods). For each session, the neural activity "convex hull" at lever pull time (i.e. t=0 sec) was defined as the hyper-volume that enclosed the t=0 sec neural activity vectors relative to all the neural activity vectors in the session (note: as convex hull analysis is sensitive to outliers a 10% K-nearest-neighbor triage was implemented prior to evaluation; see Methods). The convex hull could be visualized in two dimensions using PCA as the area enclosing the t=0sec neural activity vectors for lever pulls in that session (Fig 4d-blue dots; see also

Methods). Both the convex hull of the t=0sec and t=-1sec period prior to lever pull occupied a small subspace within the entire neural space of the session (Fig 4d-colored dots; see Methods). Going backwards in time, the convex hull space gradually increased further away from t=0sec (Fig 4e). This suggests that neural activity looks more like spontaneous (i.e. random) activity further in time from lever pulls and becomes more stereotyped towards t=0sec. The ratio of the pre-pull convex hull to the hull of the entire session was smaller than the hull computed from random trigger times (Fig 4f). Lastly, we evaluated the area under the ratio curve (AUC) longitudinally. We found that 4 of the mice (M1, M3, M5 and M6) had trends towards positive correlations between AUC and time (pearson correlation; M1: 0.58; M3: 0.24; M5: 0.27; M6: 0.48), with the remaining two mice having only very low negative correlations with time (M2: -0.06; M4: -0.12; note, only M1 and M6 had p values < 0.05). Although not statistically significant for all animals, these results point towards trends in spontaneous neural activity being potentially restructured by the task. One interpretation could be that lever pull spatio-temporal neural motifs (i.e. widefield [Ca++] spatio-temporal patterns occurring during lever pull preparation) increased their occurrence during spontaneous activity with learning, consistent with findings of occurrence increases in sensory-evoked neural motifs in mice undergoing depression paradigms (McGirr et al 2020).

Neural activity space of right paw movements and lever-pulls gradually separate. Given the findings above we sought to further evaluate systematic changes between lever-pull and random neural activity (Fig 4h-j). We recomputed convex hulls for the 1sec period prior to lever-pull, 1sec period prior to left paw and right paw movements and found that the size of the lever pull convex hulls and its overlap with the rest of the behaviors changed over time (Fig 4h). In particular, in the vast majority of mice (5 of 6) the lever pull convex hull became increasingly larger with time (Fig 4i; M1: 0.47; M2: 0.17; M3: 0.47; M5: 0.25; M6: 0.64; note: p values for mice M2, M3 and M5 were not significant, likely due to much lower number of video recorded sessions available). This data is a subset of the data above

(Fig 4g) and is largely consistent with those findings. Interestingly the intersection between the right paw convex hull, i.e. the paw used to pull the lever, and the lever pull convex hulls decreased with time in 5 of 6 mice (Fig 4j; pearson correlation; M2: -0.35; M3: -0.53; M4: -0.66; M5: -0.25; M6: -0.75) and increased with time in 1 mouse (M1; pearson correlation: 0.45; note: p values for mice M2, M3 and M5 were not significant). This finding suggests that lever pulls neural dynamics gradually become different from right paw movements despite the right paw being used for the lever pull task (note: we did not find other simpler explanations; for example, the number of lever pulls could not explain this as right-paw movements did not decrease longitudinally and most mice - M2, M3, M5 and M6 - increased the number of lever pulls longitudinally; such trends would have the effect of increasing - not decreasing - the overlap between lever-pulls and right-paw movements - yet our findings point to the opposite conclusion suggesting a neural mechanism underlies such results).

In sum, EDTs improve longitudinally in performing mice (i.e. mice with increasing numbers of lever pulls over time) suggesting neural dynamics underlying self-initiated behavior might be altered towards more stereotyped - or easier to decode - structures. The convex hull of the neural activity prior to self-initiated lever pulls grows over time yet overlaps decreasingly with right paw spontaneous movements initiations. These findings suggest that learning or mere longitudinal performance of a task restructures the neural dynamics underlying voluntary action.

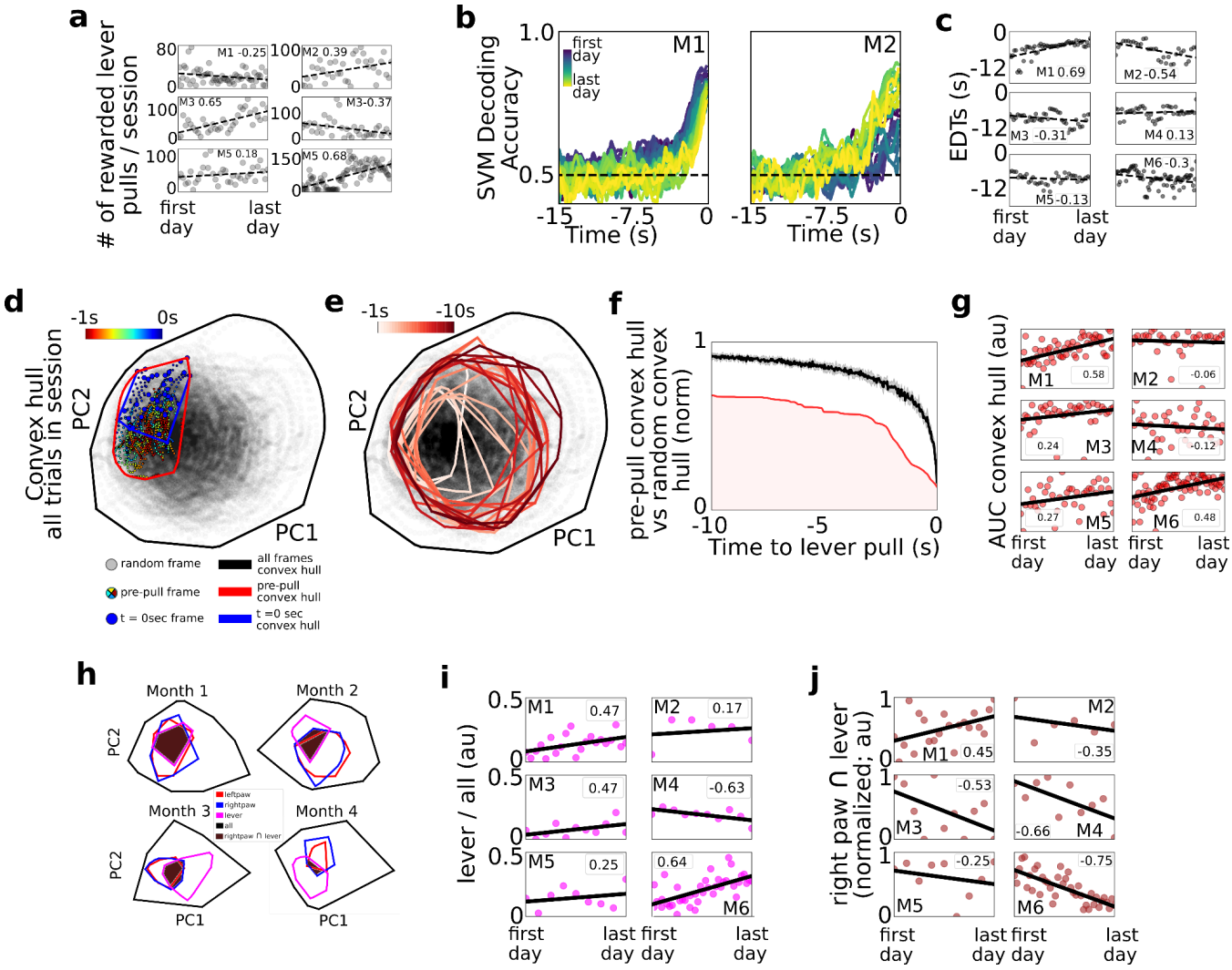


Figure 4. Tracking changes in decoding performance and spontaneous neural dynamics over time. **(a)** Number of rewarded lever pulls per session in all mice over the duration of the experiment. **(b)** SVM decoding accuracy curves from M1 and M2. **(c)** EDTs (black dots) for all mice (using concatenated trials; see Methods) show decreases in EDTs for the same 4 of 6 mice as in (a) (black lines: linear fits; inset: pearson correlation coefficient). **(d)** Convex hull of neural activity preceding lever-pulls from 30 trials from a single session in mouse M3. The colored scatter points represent neural activity at various frames in the -1sec to 0sec period and the polygons represent the convex hull of neural activity for all data (black), t = 0 sec (blue) and t= -1sec to t = 0sec (red; see also legend for details). **(e)** Same as (d) but for 1sec segments ranging from -10sec to t = 0 sec. **(f)** Convex hull volume (red line; shading represents

AUC) of pre-pulls vs. random segments (black line; shading represents 10-fold sampling standard deviation; see also Methods). **(g)** AUC (scatter points) of ratio curves (as in (f)) for all animals and sessions. **(h)** Convex hull of 1sec of neural activity preceding body movements (red and blue polygons), lever pull (magenta polygons) and all neural data (black polygons) at different longitudinal time points in the experiment for mouse M6. Brown shaded regions represent the overlap between the right paw movement initiations and lever pull. **(i)** Ratio of convex hull of lever-pull to all neural activity (magenta scatter points), linear fit (black line) and pearson correlation value (inset). **(j)** Intersection of right paw and lever pull initiation space (brown scatter points), linear fit (black lines) and pearson correlation (inset).

Fig 5. Single trial variance changes seconds prior to self-initiated lever pulls

Figure 5 here

Over the past few decades one of the most robust findings in stimulus cued decision making studies has been that stimulus onset decreases neural variability in a wide range of paradigms (see Churchland, Yu et al 2010 for a summary). This decrease in variance, also evaluated as the fano factor (i.e. the ratio of variance to mean of neural activity) has been interpreted to suggest that incoming information (i.e. stimuli) "stabilizes" the state of cortical activity (e.g. decreases the variance of membrane potential fluctuations, spiking variance or correlated spiking variability) and potentially supports the accumulation of internal memory evidence (Ratcliff 1978; Ratcliff and McKoon 2008). Here, we sought to determine whether neural activity preceding self-initiated lever-pulls exhibited a change in variance prior to the decision to pull, potentially reflecting the commencement of evaluation of "internal-state" evidence and/or the preparation of a skilled action (Fig 5).

Single trial variance decreases seconds prior to voluntary action. As shown above for single trials (Fig 3a), the average trial activity within a session becomes increasingly stereotyped near t=0sec. This stereotypy can be observed in all major cortical areas even across weeks or months of behavior (Fig 5a). Interestingly, the variance was also stereotyped with a significant change (decrease in 5 mice; increase in 1 mouse) several seconds prior to the movement related areas (e.g. motor, somatosensory and retrosplenial) but less so in areas not directly related to movement preparation (e.g. visual cortex; Fig 5b). We defined the earliest-variance-decrease-time (EVDT; see Methods) for each session as the time at which the variance decreased (or increased for mouse M2) by two times the standard deviation from a random period of time (Fig 5b - black dots; see Methods). Computing the EVDTs for all sessions and mice revealed that in most areas and animals the neural activity variance began to decrease a few to several seconds prior to lever pull initiation. While these measurements were noisy (see Methods), in mice with significant numbers of detected EVDTs (i.e. mice M3, M4, M5 and M6) the average EVDT

for all areas was around -3sec or earlier. Such decreases in variance seconds prior to behavior initiation may represent the times at which internal state evaluations and motor preparation commences (see also Discussion).

In sum, we found that in most mice and sessions, variance across all areas (excluding visual cortex) began to change several seconds prior to lever pull time. These results are consistent with and support our prior findings and could be interpreted to suggest that internal-state driven behaviors are underpinned by neural processes similar to those observed in stimulus driven decision making studies (and as observed in Murakami 2014 in neural activity preceding a self-paced task).

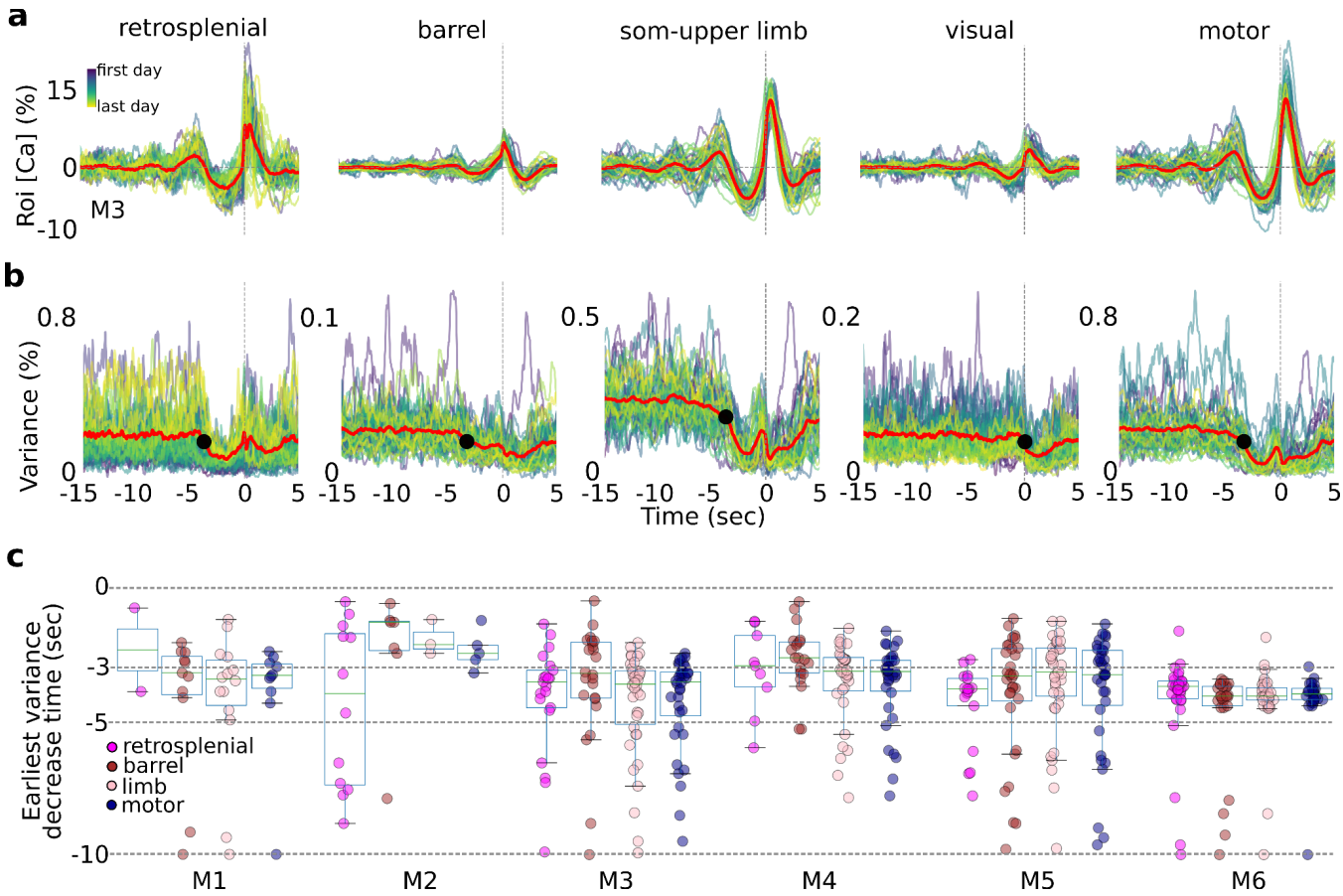


Figure 5. Internal-state evaluations begin several seconds prior to self-initiated lever pulls. (a) Average session neural activity from cortical areas (colored curves) and average over all sessions (red curve) from mouse M3. **(b)** Variance of data in (A) and the earliest variance decrease time (EVDT; black dots represent EVDT of all session averages, i.e. red curves in (a)). **(c)** EVDTs for all animals and sessions.

Fig 6. Self-caused movements have only minor effects on EDTs

Fig 6 here

An outstanding question in voluntary behavior neuroethology is how confounds, such as random body movements occurring in the period prior to a targeted action, affect behavior preparation and - in our paradigm - the decoding of future behaviors. For example, in animals that perseverate and pull the lever frequently it is not known whether decoding methods leverage dynamics from multiple lever pulls or just the lever pull occurring at t=0sec. Accordingly, we sought to evaluate the effect of excluding body movements or previous lever pulls in decoding of future lever pulls.

Rewarded lever pulls form a small portion of all voluntary actions. Across the duration of the study mice performed between 1454 (M1) to 6999 rewarded lever pulls (M6) (Fig 6a). The ratio of self-initiated rewarded and non-rewarded lever pulls, licking events, and left paw and right paw spontaneous movements for all sessions revealed that lever pulls constituted only a small portion of behaviors (Fig 6b). Across all animals the proportion of rewarded lever pulls compared to all other movements ranged from 0.03 (M1) to 0.07 (M2) (Fig 6c). Even though only selected body parts were tracked (i.e. paws and tongue), these results suggest the vast majority of the time mice are engaging in carrying out many other spontaneous behaviors as reported in other studies (e.g. Musall et al 2019).

Removing pre-action confounds by implementing a post-hoc lockout period. To evaluate the effect of intervening body movements in decoding future actions, we evaluated decoding upcoming rewarded lever pulls or spontaneous body movements preceded by periods of quiescence of varying durations (Fig 6d-h). All trials across all sessions were pooled (similar to the concatenated analysis in Fig2) and decoding was carried out after “locking-out” previous (i) lever pulls (i.e. rewarded and non-rewarded), (ii) licking events or (iii) left paw movements (note: we selected left paw movements as the right paw was used to pull the lever and excluding such movements from analysis would remove most of the rewarded lever pull trials). The number of rewarded lever pulls preceded by periods of

non-body movements or lever pulls decreased approximately exponentially with increasing lockout duration (Fig 6d). This decrease in available trials for analysis was present in all mice (Fig 6e). Importantly, locking out licking and lever paw movements beyond three seconds yielded insufficient numbers of trials for decoding analysis (see shaded region Fig 6e).

Lever pull EDTs are not affected by prior licking events. EDTs were recomputed by locking out (i.e. removing) lever pulls that were preceded by licking events in the previous 0sec, 1sec, 2sec and 3sec (note: 0sec bin removed lever pull trials that occurred exactly with a licking event based on our video recording resolution of 15FPS). We found that for all animals, the intra animal EDT distributions were not statistically different from each other (Fig 6f).

Lever pull EDTs are similar or slightly higher with increasing lever-pull lockout duration. We also recomputed EDTs for each animal and session after enforcing periods of 3-15sec of lockout (in increments of 3sec) (Fig 6g). EDTs for two mice (M1 and M2) stayed the same, while for four mice they decreased with increasing lever-pull lockout duration (M3-M6) (fig 6g). This trend was confirmed by examining the mean EDT at each lockout time point which showed in nearly all mice (excluding M2) a strong tendency for mean EDT to decrease with increasing lockout. In interpreting these results it is important to note that there were substantially fewer consecutive or same-session lever pull trials when implementing increasingly longer lockouts. Thus, *surviving* trials used for analysis came from sessions that were increasingly further apart (e.g. multiple days or even weeks). Pooling trials from separate days or weeks provides an additional source of noise due to changes in animal behavior, [Ca++] indicator and longitudinal network changes observed in our cohorts (see Fig 4). Taken all these factors into account, the results suggest that while EDTs increased slightly in some animals, the cause of the increase could not be disambiguated between [Ca++] state changes, animal behavior, systematic neural network restructuring due to longitudinal performance and the effect of pre-movement confounds (see also Discussion).

In sum, we find that despite carrying out thousands of rewarded lever pulls, such pulls constituted a small percentage of overall spontaneous body movements (likely much less if we consider other body movements we did not track). Even when pooling all trials from each animal (resulting in thousands of trials), when locking out previous licking events or left paw movements, exponentially fewer rewarded lever pulls were available with increasing locking out period. EDTs computed when excluding licking events for a period of up to 3sec did not significantly change the EDT distributions, but exclusion of previous lever-pulls up to 15sec prior to the pull slightly decreased the EDT distributions in most animals. While we cannot exclude alternative causes for this later effect (e.g. pooling imaging data across weeks or months of recording), overall these results suggest that self-caused movements play a minor role in affecting the decoding of future rewarded action.

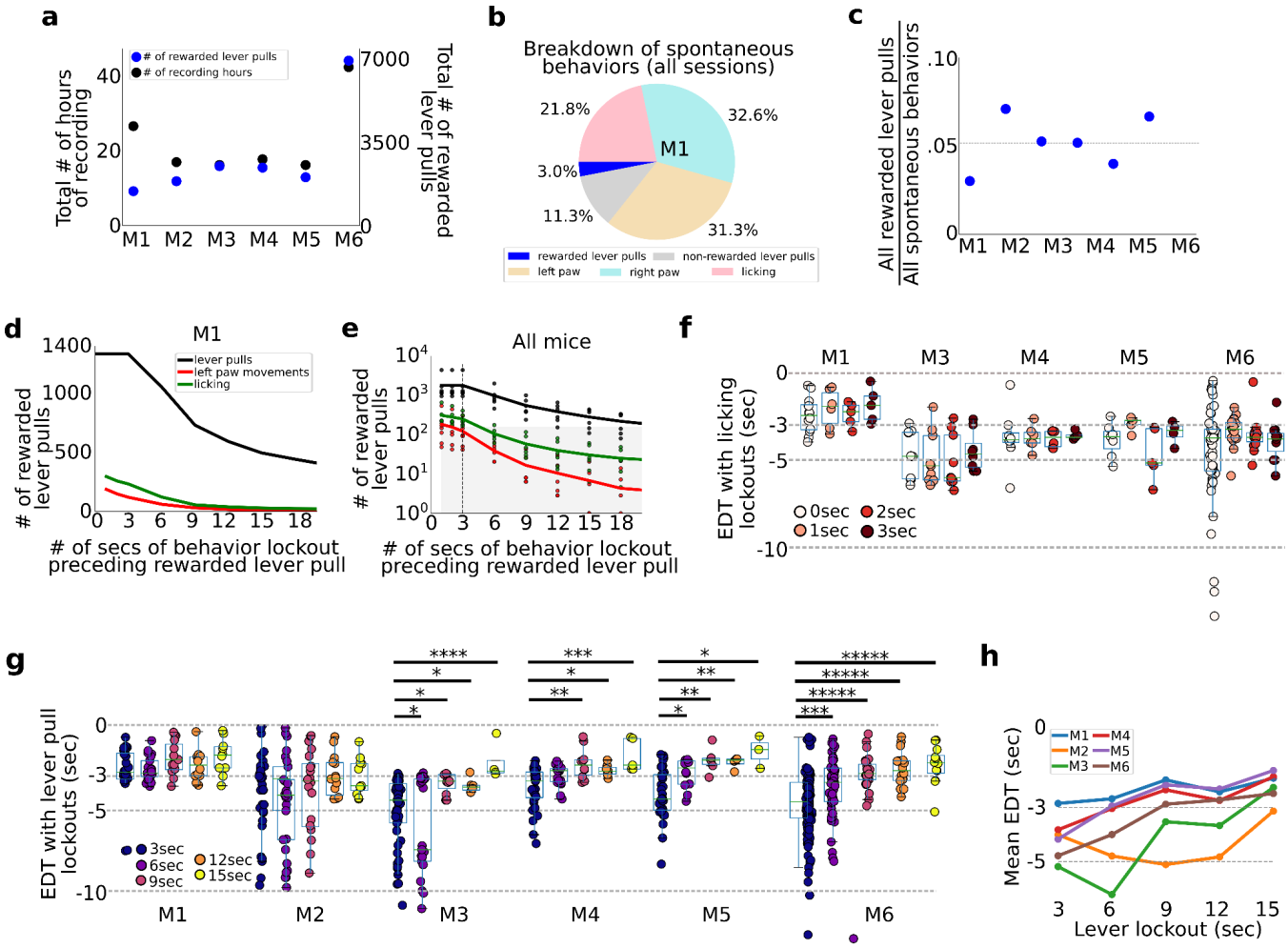


Figure 6. Tracking and evaluating the effects of self-caused movements on the decoding of rewarded lever pulls. (a) Total number of recording hours and number of lever pulls for each mouse (note each recording session was approx. 22 minutes long). (b) Percentage of rewarded lever pulls, non-rewarded lever pulls, and left paw, right paw and licking movements performed by mouse M1 across all sessions. **(c)** Proportion of rewarded lever pulls relative to all other body movements for all animals. **(d)** Number of rewarded lever pulls as a function of "locking out" previous lever pulls, licking events or left paw movements (note: locking out means excluding any rewarded lever pull trial that was preceded by a movement in the previous n-seconds; see also Main text and Methods). **(e)** Same as (d)

but for all animals and sessions (shaded region indicates lockout conditions under which less than 100 trials were present across the entire study and decoding was not carried out). **(f)** EDTs for rewarded lever pulls conditioned on licking event locking out periods of 0sec - 3sec (for clarity, the 0sec time point excluded any rewarded lever pull that occurred precisely at the same time as a licking event, i.e. to the resolution of our 15FPS video). **(g)** Same as (f) but conditioned on excluding previous lever pulls (3sec time point excluded any rewarded lever pulls that occurred exactly 3sec after a previous rewarded or unrewarded lever pull). **(h)** Mean EDTs from (g) as a function of lever pull lockout period.

Fig 7. Slow oscillations dominate pre-self-initiated behavior neural dynamics

Figure 7 here

While the role of SCP in modulating voluntary behavior is suggested in some studies (Schmidt et al 2016), less is known about the specific frequencies involved in voluntary action especially in mice. We thus sought to further characterize the frequency, power and longitudinal characteristics of slow oscillations in neural activity occurring prior to lever pulls (Fig 7).

Limb and motor cortex oscillations have most power during pre-movement neural activity. We first evaluated the power of neural activity in a session (i.e. average of neural data from all trials from -15sec to 0sec) and observed that high amplitude oscillations were present in some areas (e.g. limb cortex) but were much weaker in other areas (e.g. visual cortex) (Fig 7a). This is consistent with our self-initiated behavior task as no sensory stimuli or cues were used. This difference was consistent across all sessions with limb cortex oscillations being up to 10 times larger than those in visual cortex (Fig 7b examples from mouse M4).

Frequency and power in the average pre-movement neural activity. We evaluated the peak frequency of session averages (i.e. we computed the power-spectrum-density of the lever-pull trial average for each session; ses also Methods). Across all mice the vast majority of session averages had power peaks falling between 0.2Hz and 0.6Hz (Fig 7c). This suggests that slow oscillations dominated the pre-movement neural activity consistent with our findings that voluntary action preparation unfolds on time scales of several seconds (and consistent with the time course of our [Ca++] indicator). Turning to longitudinal trends, few statistically significant trends were present with only 3 mice showing correlations of peak frequency and time (Fig 7c; mouse M1: strong increase in peak frequencies in limb and motor cortex; mouse M2: strong decrease in peak frequencies in limb and motor cortex; and mouse M5 had an increase in peak frequency in retrosplenial cortex). The peak frequency power also exhibited differences between animals and also longitudinally (Fig 7d). For example, mouse M1 showed

significant drops in power in limb, motor and retrosplenial cortex, while other mice showed increases in power in limb or motor cortex (M2: limb; M3: motor; M4: motor; M6: limb). In sum, longitudinal changes in peak frequencies and power were minor and differed across animals.

Frequency power in single-trial pre-movement neural activity. We carried out a similar analysis as above but on an individual trial basis (Fig 7e,f). With respect to peak frequencies, distributions of frequencies from ~0.1Hz to ~0.5Hz were observed, similar to session averages. In contrast with session averages, single trial analysis showed more statistically significant trends (p values < 0.05) in most animals and areas considered (retrosplenial, limb and motor cortex): three animals had mid to strong-level increases in peak frequency (in all areas) with time (M1, M4 and M5); one animal (M6) had slight increases in frequency power in retrosplenial and limb cortex; and 1 animal has mid to strong decreases in peak frequency over time (M2) (mouse M3 had a -0.01 pearson correlation value with time in limb cortex). Peak power trends were less common, with only 2 mice showing strong correlations in the three areas longitudinally (M1 decreases in power over time; M4 increases in power over time) with the remaining mice having changes only in a single area (mouse M2 showed strong decrease in power in limb cortex; and mice M3 and M6 showed a slight increase in motor cortex power over time).

In sum, during self-initiated behavior preparation power in both session averages and individual trials was strongest in the 0.1Hz-0.7Hz. Some animals showed systematic changes in peak frequency suggesting that learning and/or longitudinal performance may change the underlying oscillatory structure of neural activity of voluntary behavior preparation. These findings suggest a complex picture with different mice potentially engaging different learning mechanisms and areas that should be considered when evaluating in-session and longitudinal performance and decoding upcoming behaviors.

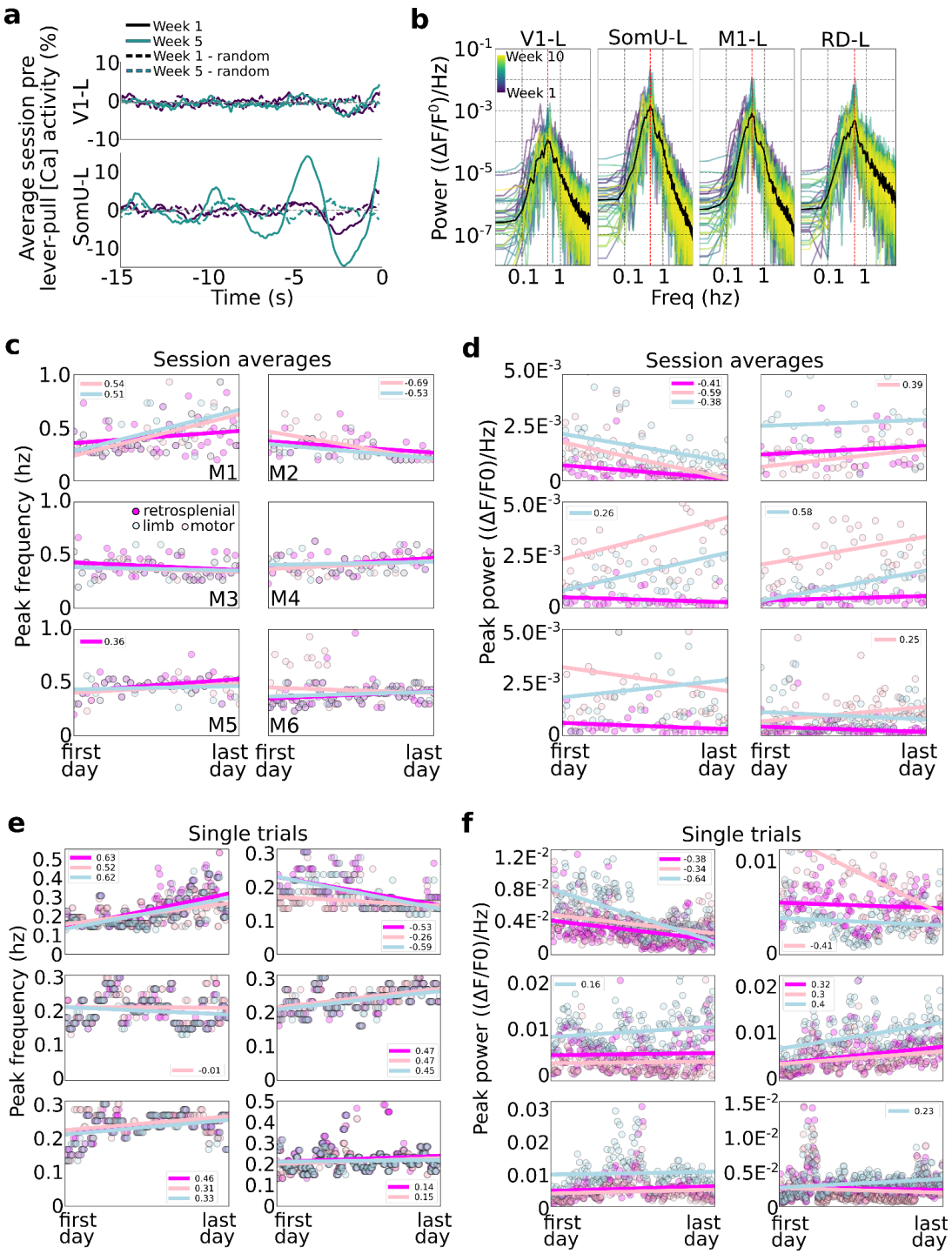


Fig 7. Slow wave oscillations underlie self-initiated behavior in mice. (a) Examples of single session averages (dark continuous curves) from two time-points and random segments (dark dashed curves) from V1-left and somatosensory-upper limb left reveal the presence of oscillations. **(b)** Power spectra of all session averages (colored curves) and average across all sessions (black curves) from mouse M4 in

four cortical areas (dashed vertical lines indicate peak of average). **(c)** Peak frequency power of each session trial average for retrosplenial, motor and limb cortex (colored scatter points). **(d)** Same as plot in (c) but for peak power for all animals and sessions. **(e)** Same peak frequency analysis as in (c) but for single lever pull trials (instead of session averages). **(f)** Same peak power analysis as in (d) for single trials.

DISCUSSION

Since the 1960s, several human neuroscience studies seeking to identify the neural correlates and genesis of self-initiated, voluntary action have found that increases in neural activity in SMA and preSMA precede both voluntary movement and even awareness of the intent to act (Kornhuber and Deecke 1965; Libet et al 1983). Most studies found only small differences (~150 ms) between the intent to act and voluntary action initiation; however, these findings remain controversial and determining the precise arrival of subjective intent and the effect of reporting it is a complex topic (see e.g. Wegner 2002; Dijksterhuis et al 2006; Tusche et al 2010; Sinnott-Armstrong 2010; Dijksterhuis and Aarts 2010). Removing the reporting of intent from voluntary behavior paradigms and focusing solely on the relationship between neural activity and self-initiated action avoids some of the controversies and focuses the debate on the study of objective variables (e.g. timing of behavior initiation, neural activity in specific areas) - and enables the use non-human animal models for voluntary action research.

Voluntary actions in mice are prepared seconds prior to movement and are biased to occur during specific phases of slow oscillations. Using a self-initiated voluntary behavior paradigm in mice to relate pre-movement neural activity to the initiation of behavior enabled us to collect a high number of behavior trials across weeks and months of recording and with higher neuroanatomical resolution recordings than EEG and higher temporal resolution than fMRI. Self-initiated behaviors in mice were preceded by an increase in neural activity starting a few to many seconds prior to - and peaking at - behavior time, similar to the EEG RP signal in humans (Fig 1). We further found that decoding of upcoming behavior a few seconds to more than ten seconds prior to movement was possible, a finding consistent with and expanding on findings in humans (Soon 2008; 2013; Bode et al 2011; see Figs 1,2). Self-initiated voluntary behaviors were even more biased towards specific phases of neural activity than in humans (Fig 3). This suggests that in mice, oscillation phase could be even more determinative of action initiation timing than in humans. Overall, these results link human voluntary action studies with

rodent self-initiated behavior studies and suggest mice as an adequate model for studying the neural correlates of self-initiated action.

Behavior preparation signals are distributed across the cortex. While the vast majority of voluntary action studies in humans focused on SMA (and preSMA), we found that decoding future behaviors using motor (or limb cortex) was inferior to decoding using all cortical activity (Fig 2). This suggests that pre-voluntary movement neural signals are widely distributed across the dorsal cortex but also that more direct neural recordings may be required for a complete characterization of pre-movement neural activity in humans (and non-humans). Coupled with a neural recording modality that more precisely reports local neural activity (i.e. widefield [Ca++] cortex) our findings suggest that voluntary action studies in humans may improve if subcranial neural signals from multiple areas were available.

Longitudinal performance of a voluntary task restructures the neural dynamics underlying voluntary action and spontaneous neural activity. It has been suggested that noise-driven stochastic models (i.e. leaky stochastic accumulators) can explain the RP as a result of averaging backwards in time over multiple stochastically determined behavior initiations (Schurger et al 2012). In contrast, a recent human study aimed at testing part of this hypothesis found that the RP amplitude increased with learning suggesting that the RP represents planning and learning rather than stochastic structures alone (Travers et al 2021). Consistent with the later study, we also found that decoding of upcoming water rewarded lever pulls improved in most mice, specifically, those who increased their performance with time after weeks of behavior (see Fig 4). We also found structural changes in the neural dynamics as both the lever pull neural space and its overlap with the left-paw changed systematically with time. These results suggest that learning, or merely longitudinal performance and betterment, of a high-value behavior (e.g. water seeking in water deprived mice) increases stereotypy even in self-initiated actions. These large-scale cortical changes in brain activity have been observed in humans, for example, while learning a brain-machine-interface task (e.g. Wander et al 2013) but aside from a very recent EEG study

(Travers et al 2021) are not well described. The mesoscale mechanisms for such changes may involve increased representation of lever-pull preparation dynamics in the overall spontaneous dynamics (e.g. occurring more frequently) or increase differentiation between lever-pull preparatory neural activity and other behaviors. These results add to the evidence that voluntary actions are supported by learned neural-dynamical structures and that those can change on longer time scales.

Internal state evidence accumulation commences seconds prior to movement. We found that both lever-pull decoding accuracy increases nearly monotonically with approaching action (e.g. Fig 4b) and that intra-session variance decreases several seconds prior to movement within limb, motor and retrosplenial cortex. We suggest these findings constitute further "indirect evidence that evidence-accumulation" (Boid et al 2014) is occurring even in the absence of explicit stimuli - likely based on evaluations of internal states and models. This supports the hypothesis that internal-state driven self-initiated actions could be potentially modeled by commonly used perceptual and cognitive decision making models (Gold and Shadlen, 2007; Heekeren et al. 2008; Murakami et al 2014).

Self-caused movement confounds have only minor effects on the decoding of future rewarded action. Our study does not directly address the effects of stimuli or other information on the neural dynamics preceding self-initiated decisions, for example, as in some human choice paradigms that consider decision choice in the presence of novel information (e.g. Maoz et al 2019). However, considering the effects of intervening self-caused spontaneous actions (e.g. lever pulls, licks and body movements) we found they had only minor effects on decoding accuracy. This suggests that internal-state driven (i.e. uncued) decision making processes may not be strongly affected by contemporaneous body movements.

Inter-animal variability. We found inter-animal differences in many format: different decoding times, anatomical areas involved as well as longitudinal dynamics (e.g. differences in longitudinal decoding trends Fig 4; or preferred phase angles at behavior time different Fig 3; different trends in

frequency peak and power Fig 7). These results suggest that neural dynamics and strategies for initiating a voluntary action could be specific to individuals and that pooling over multiple subjects may remove novel or important nuances. In other words, single subject analysis may be critical to further advancing the debate on pre-voluntary action dynamics.

Limitations. Our study focused on characterizing *neural dynamics* and *timing* of an ethologically valuable movement rather than identifying *intent* or *awareness* of upcoming movements. As such, we do not directly address the role of subjective "intent" as in some human EEG studies (e.g. Libet et al 1983) or the role of reasons or deliberation on decision making (Note: as mentioned above, the effect of reporting intent and the use of reasons or deliberation in voluntary actions are the subject of ongoing debates; see, for example, Dijksterhuis et al 2006; Dijksterhuis and Aarts, 2010; Vierkant et al 2019; Wegner et al 2002). Although our study was not aimed at disambiguating between the timing of intent awareness and movement, a reasonable assumption is that mice form the intent to act near to or coinciding with the lever pull time. Uncued voluntary action studies in humans generally find the difference between the timing of subjective intent and movement to be small (e.g. 150ms; Libet et al 1983) or even negligible, orders of magnitude smaller than the EDTs in our results. Second, our decoding times showed a strong dependence on the *number of trials* suggesting that additional trials would change (most likely improve) our decoding results. Although it is challenging to keep animals motivated across many trials within a single ~20min session, decreasing reward size might have increased the number of self-initiated lever pull behaviors and reduced the dependence we observed. Third, we sought to remove pre-movement confounds from our results by "locking out" previous lever-pulls or body movements (Fig 6). A more direct approach where animals are specifically trained to remain quiescent prior to an action may yield more trials and easier to interpret results. However, it is practically challenging to train mice to withhold behaviors for significant periods of time (e.g. >> 3sec) while also performing a task for a valuable reward. Fourth, we found strong correlations between lever

pulls and body movements in all mice (not shown). However, we did not take into account the temporal location of lever pull activity when decoding the body movement (Fig 2); for example, we decoded upcoming left paw movement initiations without accounting for - or removing - lever-pull invitations co-occurring with such paw movements. It is obvious that many of the spontaneous paw movements also coincided with lever-pulls and thus the EDTs for paw movements were not an independent measure from the EDTs of rewarded lever-pulls. However, we chose to remain agnostic and not separate body movement initiations into those coinciding with lever pulls and those that occurred many seconds away from rewarded lever pulls (this also had the effect of preserving a higher number of body-movement trials for decoding). Despite not separating the data, we did find a significant difference in lever pull and right paw dynamics longitudinally (Fig 4j) suggesting that further separation may have only increased this difference. We acknowledge that it would have been interesting to divide the behaviors and carry out separate analysis, and leave this direction for future projects.

Conclusion. Over the past few decades rodent models of sensory systems and decision making have become increasingly common (e.g. visual evidence accumulation; Najafi and Churclhand 2018; Odoemene et al 2018; IBL et al 2021). The findings presented here suggest that mice could also be an appropriate model for neuroscience investigations into self-initiated action. While characterizing the dynamics underlying self-initiated behavior in rodents advances our understanding of voluntary action it could also advance our understanding of developmental and psychiatric disorders that have behavioral symptoms such as avolition in depression (e.g. lack of will to move; Brakowski et al 2017) and behavior repetition observed in obsessive-compulsive-disorders (Lysaker et al 2018). Our findings suggest that the neural mechanisms underlying voluntary action preparation and performance could be preserved in part, or in whole, between humans and mice and that studies of voluntary action would benefit from mouse models and the vast libraries of behavior, genetic and neural recording methodologies available.

MATERIALS & METHODS

Mice. Mouse protocols were approved by the University of British Columbia Animal Care Committee and followed the Canadian Council on Animal Care and Use guidelines (protocols A13-0336 and A14-0266). Six GCaMP6 transgenic male mice (Ai93 and Ai94; Madisen et al 2015) were used. For the study the mice names were defined as M1-M6 and had the following genotypes: M1 - M5: Ai94; M6 - Ai93.

Lever pull task. Mice were kept on a restricted water schedule as previously described (see Silasi et al 2016). Briefly, mice were implanted with a head post and head fixed with their bodies partially resting in a 28mm diameter Plexiglass tube. A 1cm cutout from the right side of the tube floor accommodated a monitored lever that was positioned at the same height as the tube. At the start of each session a water spout was set near the mouth of the mice, ensuring the mice could obtain dispensed water drops by licking. In order to receive a water reward mice were required to pull the lever past a threshold and then hold the lever without pulling it to the maximum value. Correct lever pulls - "rewarded lever pulls" - were tracked in real time to provide water reward. Following a reward a lockout period of 3 sec was implemented during which mice could pull the lever but would not be rewarded irrespective of performance. This task required learning the minimum threshold, the duration of the hold and the refractory period of the lockout. We selected 3 seconds as longer lockout values limited the consistent acquisition of the lever pull task. We recorded widefield calcium activity across each recording session (1330 sec, i.e. ~22mins long) over many days (42-109 days, average = 58.3 +/- 24.6 standard deviation). Mice had longitudinal trends with some increasing the # of lever pulls over time and others decreasing (See Fig 4a; M1-M6: pearson correlations: -0.25, 0.39, 0.65, -0.37, 0.18, 0.68; p values: 0.048, 0.020, 1.095e-5, 0.288, 6.320e-15; Note: because mice were not habituated we discarded the first week of training in this computation to better capture longitudinal trends rather than habituation idiosyncrasies).

Widefield Calcium Imaging. Widefield calcium imaging was carried out as described previously (Xiao et al 2017; Silasi et al 2016). Briefly, mice with a chronically implanted transcranial window were head fixed under a macroscope. Images were captured at 30 Hz with 8x8 pixel binning, producing a resolution of 68µm/pixel (Vanni and Murphy, 2014). To visualize the cortex, the surface of the brain was illuminated with green light (but not during image acquisition). Calcium indicators were excited with blue-light-emitting diodes (Luxeon, 470 nm) with bandpass filters (467–499 nm). Emission fluorescence was filtered using a 510–550 nm bandpass filter or collected in a multi-band mode as described below. For single wavelength green epifluorescence, we collected 12-bit images at varying time resolution (33 ms; i.e., 30 Hz) using XCAP imaging software. In order to reduce file size and minimize the power of excitation light used, we typically bin camera pixels (8 × 8) thus producing a resolution of 68 µm/pixel. Hemodynamic correction was not available as we only used single wavelength excitation. Based on previous experiments using similar imaging conditions (Vanni and Murphy 2014, Silasi et al. 2016, Vanni et al. 2017) in control GFP expressing mice we would not expect significant contributions from hemodynamic signals under the conditions we employed. These imaging parameters have been used previously for voltage-sensitive dye imaging (Mohajerani et al., 2013) as well as anesthetized GCaMP3 imaging of spontaneous activity in mouse cortex (Vanni and Murphy, 2014) and awake GCaMP6 imaging in mouse cortex with chronic window (Silasi et al 2016).

Behavioral recordings. Behavior was recorded using a Windows OS camera at 15 frames per second. Video recordings were saved in the native .wmv format and converted at the same resolution to .mp4

format for post-processing steps. Each video recording session lasted approximately 22 minutes and contained ~20,000 frames.

$\Delta F/F_0$ computation. $\Delta F/F_0$ computation was carried either via bandpass filtering (0.1Hz to 6.0Hz) or as previously described (Xiao et al 2017). Briefly, F_0 was computed as the average pixel activity in the window preceding the analysis window. For example, for analyses of neural activity within a +/-3sec window following a behavior, F_0 was computed based on the previous 3 sec of neural activity, i.e. the -6 sec to -3 sec window. We found no statistical differences in our results between using sliding window $\Delta F/F_0$ or bandpass filtering and for our analysis we relied only on bandpass filtered data.

Registration to Allen Institute dorsal cortex map. We used a 2D projection of the Allen Institute dorsal cortex atlas, similar to Musall et al., 2019, Saxena et al., 2020 and Couto et al., 2021 to agnostically identify ROIs without the need for stimulus driven or other neuroanatomical markers. We rigidly aligned the widefield data to a 2D projection of the Allen Common Coordinate Framework v3 (CCF) (Oh et al., 2014) as in Musall et al., 2019, Saxena et al., 2020 and Couto et al., 2021, using four anatomical landmarks: the left, center, and right points where anterior cortex meets the olfactory bulbs and the medial point at the base of retrosplenial cortex. The ROI's identified for each animal and session were individually inspected to qualitatively match expected activation of somatosensory cortex during lever pull trials in each session.

Analysis. All analysis was carried out using custom python code developed as part of an electrophysiology and optical physiology toolkit available online (https://github.com/catubc/widefield). Methods for computing event triggered analysis for widefield imaging have been previously published (Xiao et al 2017) and are also available online (https://github.com/catubc/sta_maps).

Unsupervised behavior annotation and body movement computation. Seven features were identified for tracking: center of left paw, center of right paw, the underside of the jaw, the tip of the nose, the underside of the right ear, the tongue and the midpoint of the lever. DeepLabCcut (DLC v. 2.1.8; Mathis et al 2018) was used to label these features in 60 frames per video for 3 videos in each animal. The DLC predictions were inspected and smoothing was applied to correct missing or error frames (using a 30 frame window sliding mean or Savitsky-Golay 31 frame filter using 3rd degree polynomial; note mouse M2 did not have good tongue tracking and this feature was excluded from analysis). Body movement initiations were computed as the first time point at which the velocity was larger than 3 times the standard deviation of velocity over all periods. We then excluded movement initiations which were preceded by another initiation (of the same body part) in the previous 3 sec of time.

Principal Component Analysis. PCA was applied to neural activity time series to decrease the dimensionality and denoise the data. For each session we first converted the filtered [Ca++] neural activity from pre lever neural recordings from time -15 sec to + 15 sec into a series of [n_frames, width_pixels * height_pixels]. This data was then run through principle complement analysis linear dimensionality reduction using the python sklearn package to obtain a pca model (https://scikit-learn.org/stable/modules/generated/sklearn.decomposition.PCA.html). We next selected the number of principal components required to reconstruct the data to >= 95% variance explained precision. Lastly, we applied the PCA model (i.e. denoised) to both the lever-pull neural data and control data.

Support vector machine classification - decoding single sessions. We used SVM classification to decode neural activity preceding an action vs random periods of time using methods similar to those

used in humans with functional Magnetic Resonance Imaging data (fMRI; Soon et al 2008). Briefly, for each session and each rewarded lever pull or body movement initiation we extracted segments of neural activity 30sec long centered on the time of the action (i.e. -15sec to +15sec following the action). Controls were selected similarly but the time of the action (i.e. t=0sec) was randomized to fall anywhere in the session except a +/-3sec window around an action. For clarity, controls could contain neural activity from rewarded lever pulls or body initiations; we found this to be a more conservative method than to manually select only non-movement periods as controls. We next denoised both the behavior data and the control data using PCA (see description above). We then built SVM classifiers using as input 1 sec-wide windows (30 frames @ 30 FPS) of data from both the behavior (i.e. class #1) and the random controls (i.e. class #2). The input to each SVM classifier was a 2D array [n_trials, n_frames * n_PCs]. For example, in a session where >95% of the data dimensionality was captured by 10 PCs, the input the the SVM classifier was: [n_trials, 30*10] = [n_trials, 300]. We similarly computed the control array and the SVM classifier was trained on two classes (i.e. lever pull vs. control). We tested additional sized windows (i.e. single frame=30ms, or 150 frames = 5 sec) but did not see significant improvements. We used sigmoidal SVM kernels as they showed a slight improvement in SVM accuracy over linear kernels (see https://scikit-learn.org/). We carried out 10 fold cross validation using a split of 0.9:0.1 train:validate. The output of the SVM classification for a 1sec window was assigned to the value of the last time point in the stack (e.g. the accuracy computed from decoding the -15sec to -14sec time window was assigned to the -14sec time bin). We carried out this SVM classification for each time point in the -15sec to +15sec window. For clarity, 870 SVM classifiers (-15 sec to +14 sec = 29sec *30 fps = 870 frames) were trained for each validation point (i.e. 10- fold cross validation).

Support vector machine classification - decoding concatenated sessions. We additionally trained SVM classifiers on concatenated sessions to increase the number of trials available. Sessions were

concatenated across sequential behavior days to reach a minimum of 200 trials. Intra-session PCA was first applied to each session to denoise the data locally (using a minimum of 95% reconstruction accuracy as above. The denoised time series were then concatenated and fit to a PCA model (https://scikit-learn.org/stable/modules/generated/sklearn.decomposition.PCA.html) using randomly sampled (3%) of the data from the concatenated stack. The multi-session PCA matrix was then used to denoise the individual sessions and we kept a fixed 20 principal components to reconstruct the concatenated datasets. The remaining steps (SVM training and decoding) were carried out as for the single session approach described above.

Computation of Earliest Decoding Time (EDT). We sought to use a method that detected the first time point in the cross-validated SVM accuracy curves that was above chance at a statistically significant level (i.e. student t-test p value < 0.05). We denoted this time as the Earliest-Decoding-Time (EDT) for each session. We obtained the EDT for each session using several steps. First, for each session, we computed the 10- fold cross validated accuracy curves using 30-time step (i.e. 1sec) windows (as described above but we additionally filtered the accuracy curves using a 30-time step moving average to further decrease the effects of noise on the prediction curves. Next, we obtained the significance at each time point by computing a 1-sample t- test between the SVM cross validation accuracy values (i.e. 10 values) and a population mean of 0.5 (i.e. chance) using the python scipy stats package. We next applied a Benjamini-Hochberg correction for multiple tests using the python statsmodel package. Finally, starting at t=0 sec, we moved backwards in time until we found the last time point that was statistically significant (i.e. p value < 0.05 as computed above). This last step had the effect of imposing a constraint which required all decoding accuracy distribution times following the EDT to be statistically significant - thus excluding random stochastic fluctuations in the accuracy curves which could result in very low EDTs that are not reasonable or meaningful. The effects of this last constraint could be observed in Figs

2F,G where the EDT is higher (i.e. closer to t=0sec) than other time points that are statistically significant (see top colored bars for statistical significance and note that there are some isolated times that show statistical significance).

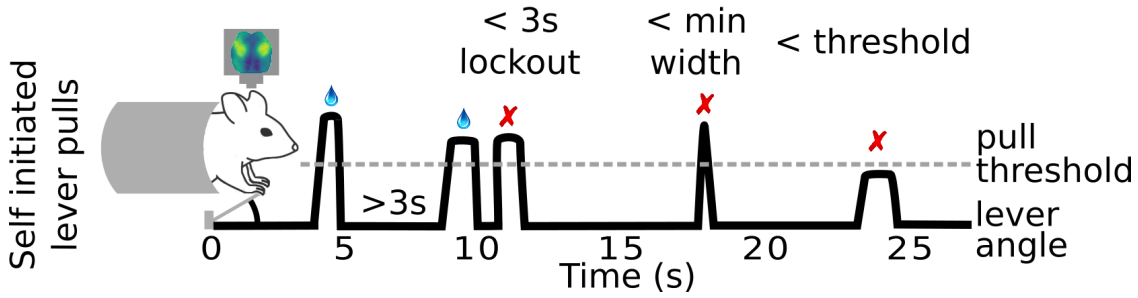
LocaNMF: LocaNMF was applied to the data from each widefield session as in Saxena et al., 2020. Briefly, we applied semi non-negative matrix factorization (sNMF) to the denoised data, while encouraging localization in the spatial components to the Atlas regions. This results in spatial components that are aligned to the different Atlas regions, thus allowing us to further analyze the corresponding temporal activity in each region. The locaNMF parameters used were as follows: maxrank = 1; min_pixels = 200; loc_thresh = 75; r2_thresh = 0.96; nonnegative_temporal = False; maxiter_hals = 20; maxiter_lambda = 150; lambda_step = 2.25; lambda_init = 1e-1.

Power spectra. LlocaNMF temporal component spectra was computed using the python scipy signal package.

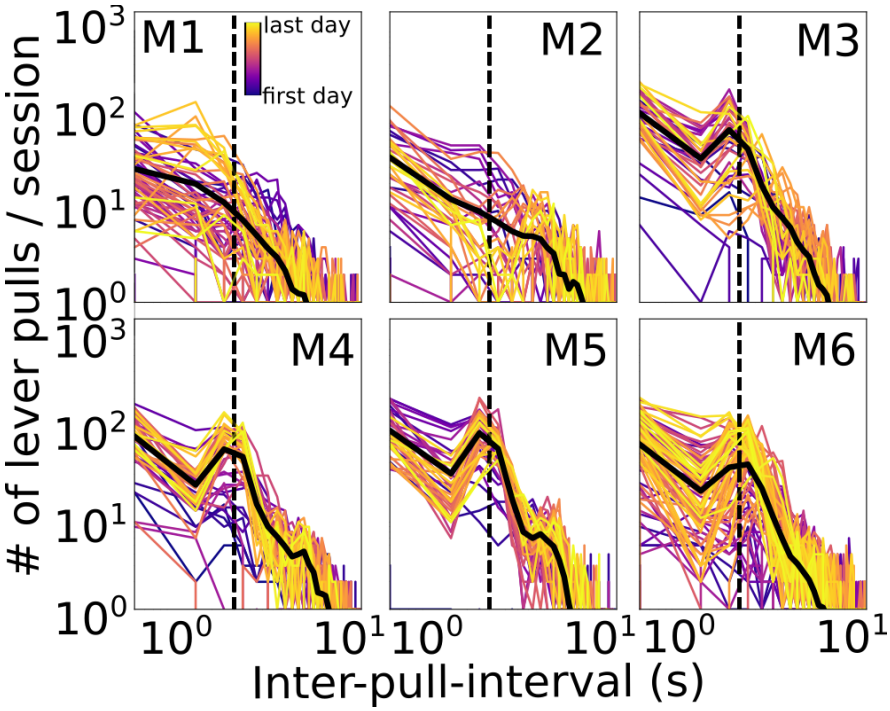
Pre-movement region-basedROI phase computation. We computed the phase of a neural activity of single trial for each region's temporal componentsROI by fitting a sine function to the period of -5 sec to 0sec prior to lever pull. We next computed the phase of each trial as the intersection of the sine fit with the t = 0 sec line.

Sinusoidal fits to single trial data. Sinusoids were fit using scipy curve fit function to single trial neural data from each area based on the last 5 seconds preceding the lever pull (i.e. -5sec..0sec)

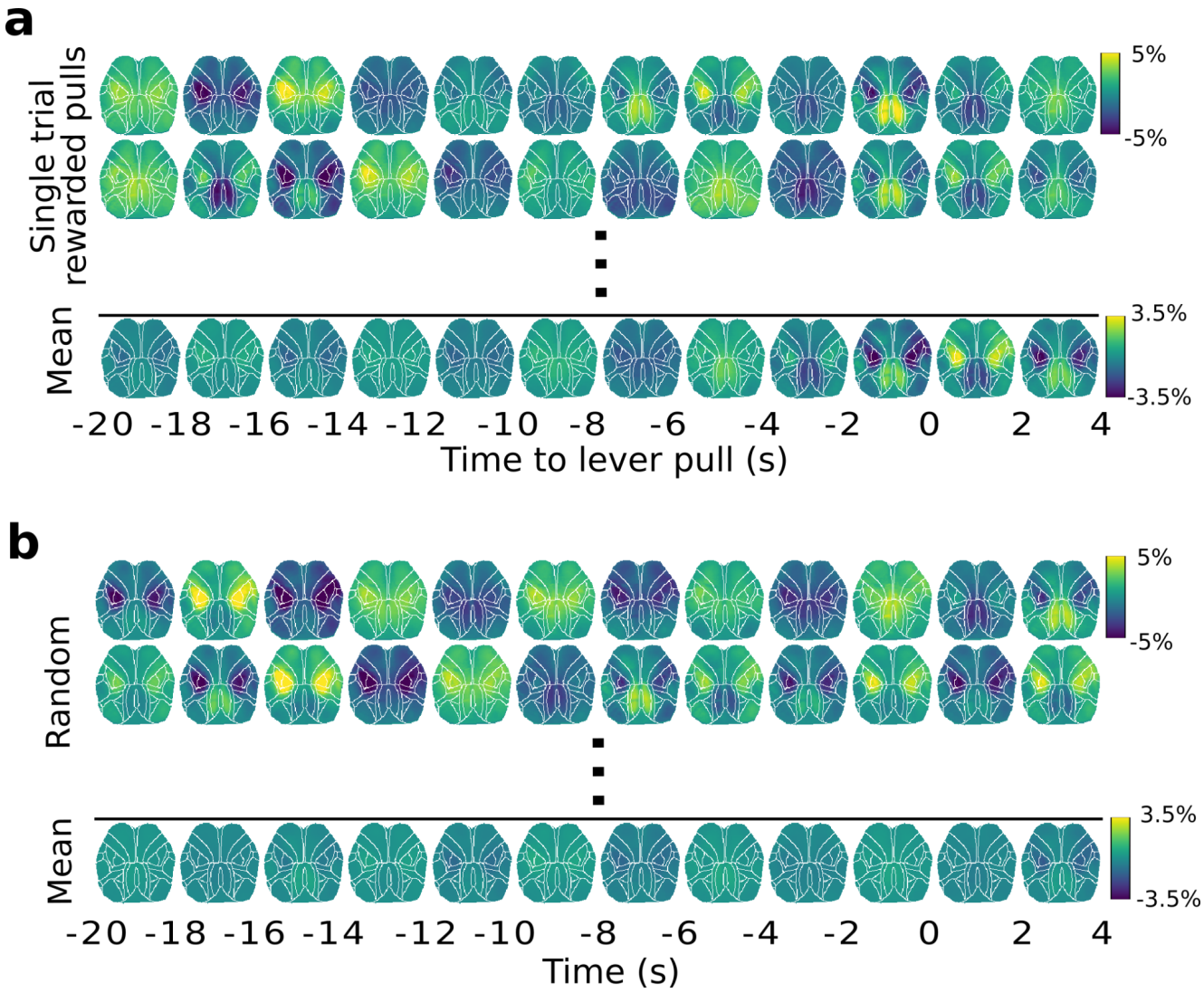
Earliest variance decrease time (EVDT). We defined the earliest variance decrease time EVDT for each session as the time at which the variance in a 1sec sliding window decreased by 2 x the standard deviation of the variance computed in the window -30sec to -15 sec prior to the lever pull. We found the requirement for all variance values in a 1sec sliding window to fall below the threshold as necessary to deal with noise or fluctuations in variance. We also required the EVDT to fall between -10sec and 0.5 sec prior to the pull or it was discarded from analysis resulting in many sessions being discarded from analysis, especially for mice M1 and M2. More robust methods for detecting the variance decrease are likely possible, but were not explored. We also note that in Mouse M2, the variance change prior to movement was positive (i.e. variance increased) and thus we used the absolute of the difference to compute the EVDT (instead of only considering decreases).



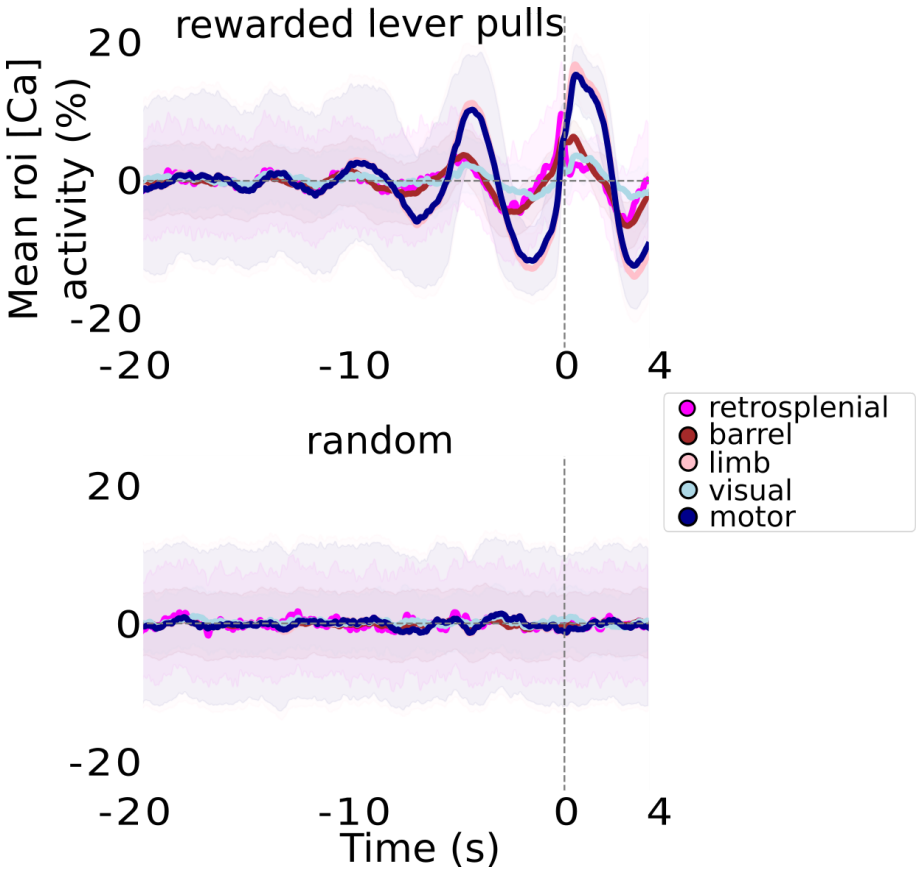
Supplementary Fig 1.1: Mouse self-initiated lever pull paradigm. Mice are trained to pull a lever to receive a water reward. In order to receive a water reward the lever pulls must occur at least 3 seconds after a previous lever pull, pass a minimum lever angle threshold, and the lever must be held past the threshold for a minimum amount of time (e.g. 0.1sec).



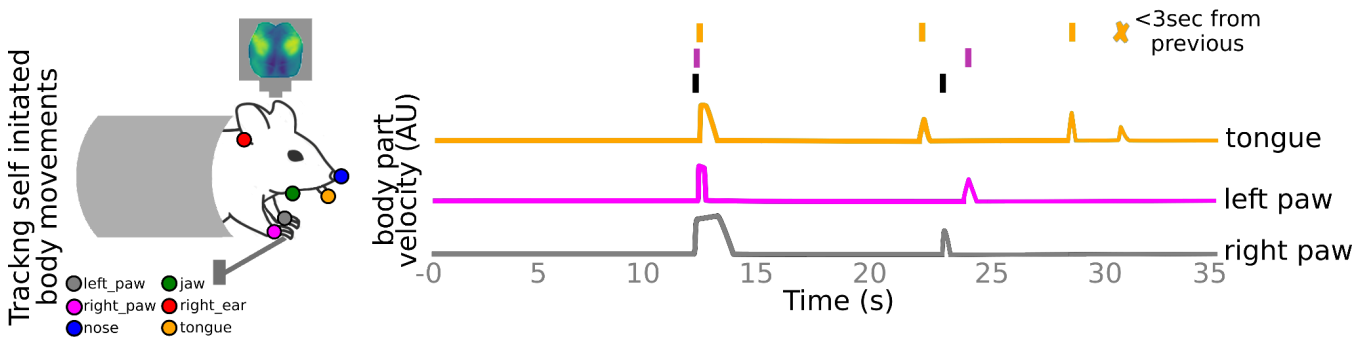
Supplementary Fig 1.2: Mouse longitudinal inter-lever-pull interval distributions. Inter-lever-pull distributions for each session (colored lines) and all session averages (black lines) for all mice. Dashed line indicates t=3sec which was the minimum "lockout" time (i.e. no previous lever pull) in order to receive a water reward. (Lever pull times were defined as the initial movement of the lever from rest; see Methods).



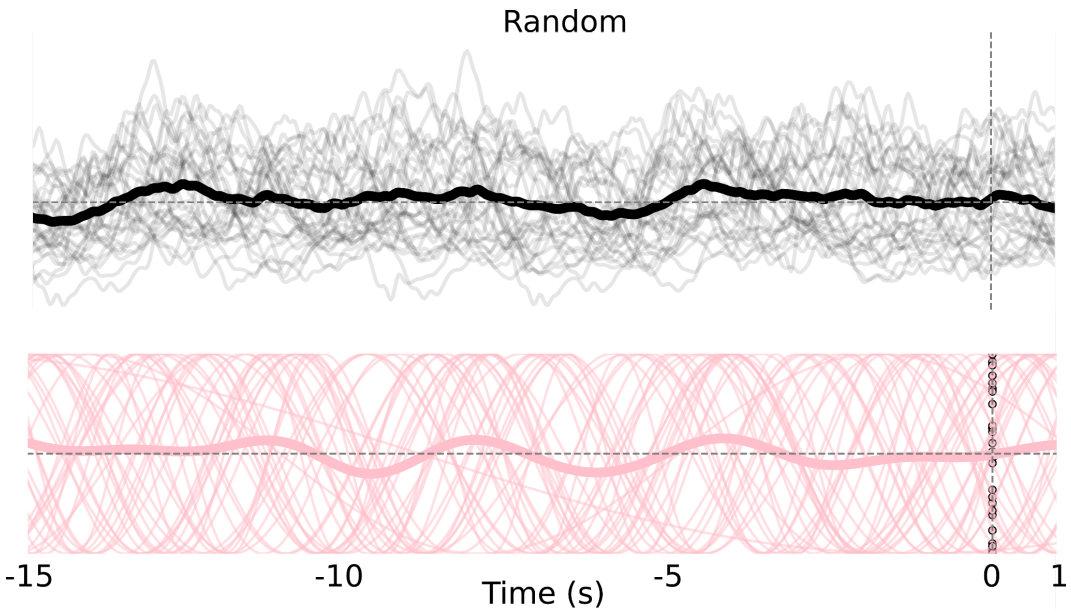
Supplementary Fig 1.3: Widefield [Ca++] dynamics during random vs. rewarded lever pulls. (a) Top: widefield [Ca++] activity from single lever pull trials; Bottom: widefield [Ca++] activity obtained for a session average (30 trials). **(b)** Top: same as (a)-Top but for a random segment of neural activity (i.e. not locked to lever pull at t=0sec); Bottom: same as (a)-Bottom but for random neural segments.



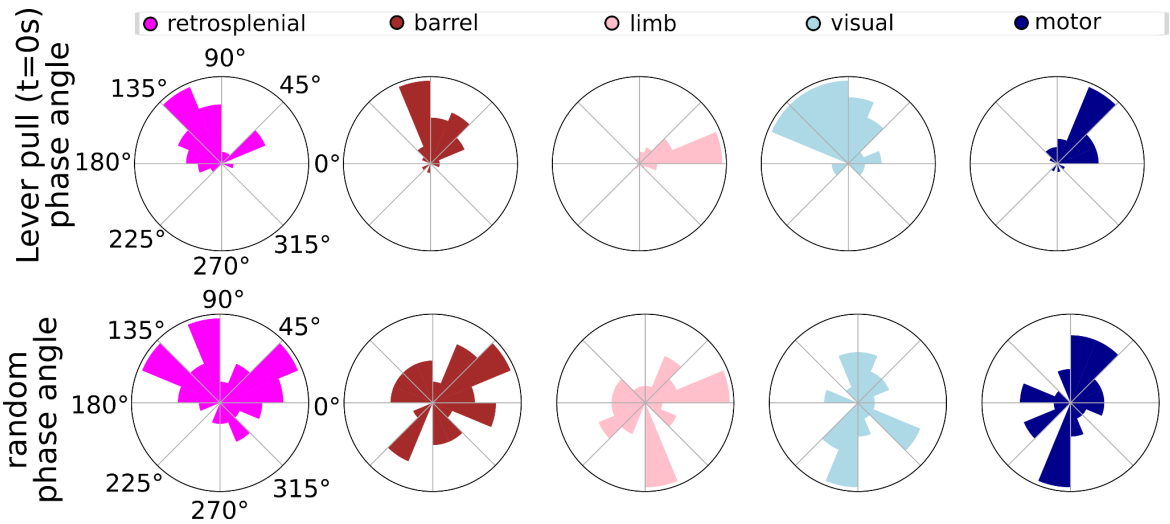
Supplementary Fig 1.4: ROI-specific widefield [Ca++] dynamics during random vs. rewarded lever pulls. (a) Top: session average widefield [Ca++] activity from five cortical areas during a rewarded lever pull; Bottom: same as Top but for random segments of neural activity.



Supplementary Fig 2.1: Tracking self-initiated body movements. Markerless pose estimation methods are used to track the locations of left paw, right paw, nose, right year, jaw and tongue. Self-initiated body movements are defined as increases in body part velocities above noise threshold and not preceded by a body movement < 3 sec prior (see also Methods).



Supplementary Fig 3.1: Random controls for slow oscillation sinusoidal fits. Top: single trial neural activity from left somatosensory (limb) cortex (thin black lines) from a session and session average (thick black line). Bottom: sinusoidal fits to data in Top (thin pink lines) and session average (thick pink line).



Supplementary Fig 3.2: Phases for lever pulls vs. random segments across major cortical areas. Top: single trial phase distributions for retrsponelina, barrel, limb, visual and motor cortex during rewarded lever pull trials in a single session. Bottom: same as Top but for random time points.

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Table 1. Lever pull statistics

Animal ID	# of behavior sessions*	# of video sessions*	Median # rewarded pulls / session	Median # unrewarded pulls / session
M1	69	30	16	14
M2	42	12	34	14
M3	42	11	42	135
M4	46	10	33	111
M5	42	11	38	116
M6	109	70	45	35

* Total numbers reported include also rejected sessions due to insufficient trials (see also Methods).