Circuit basis for functional heterogeneity of principal neurons in sensory cortex

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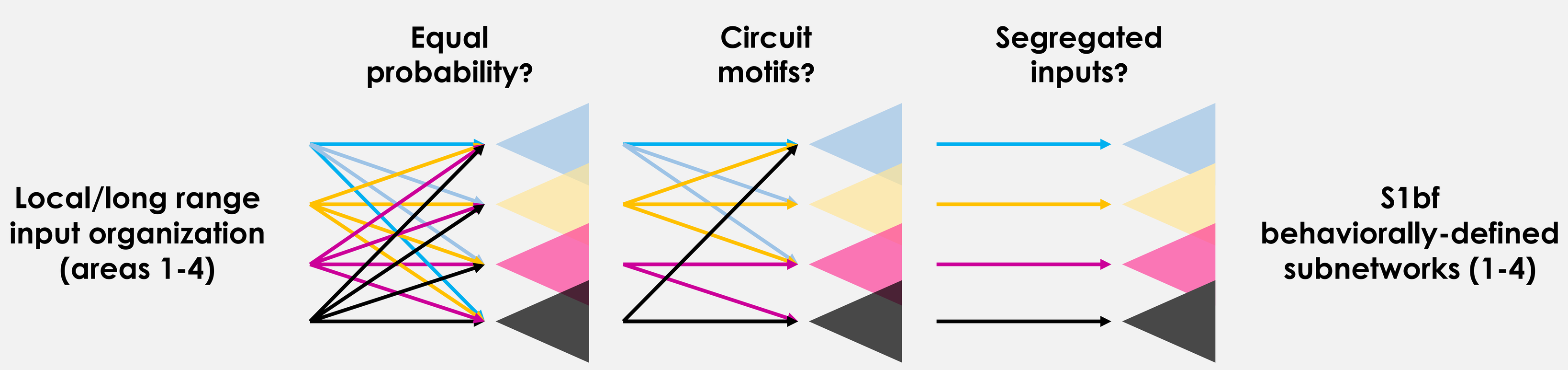
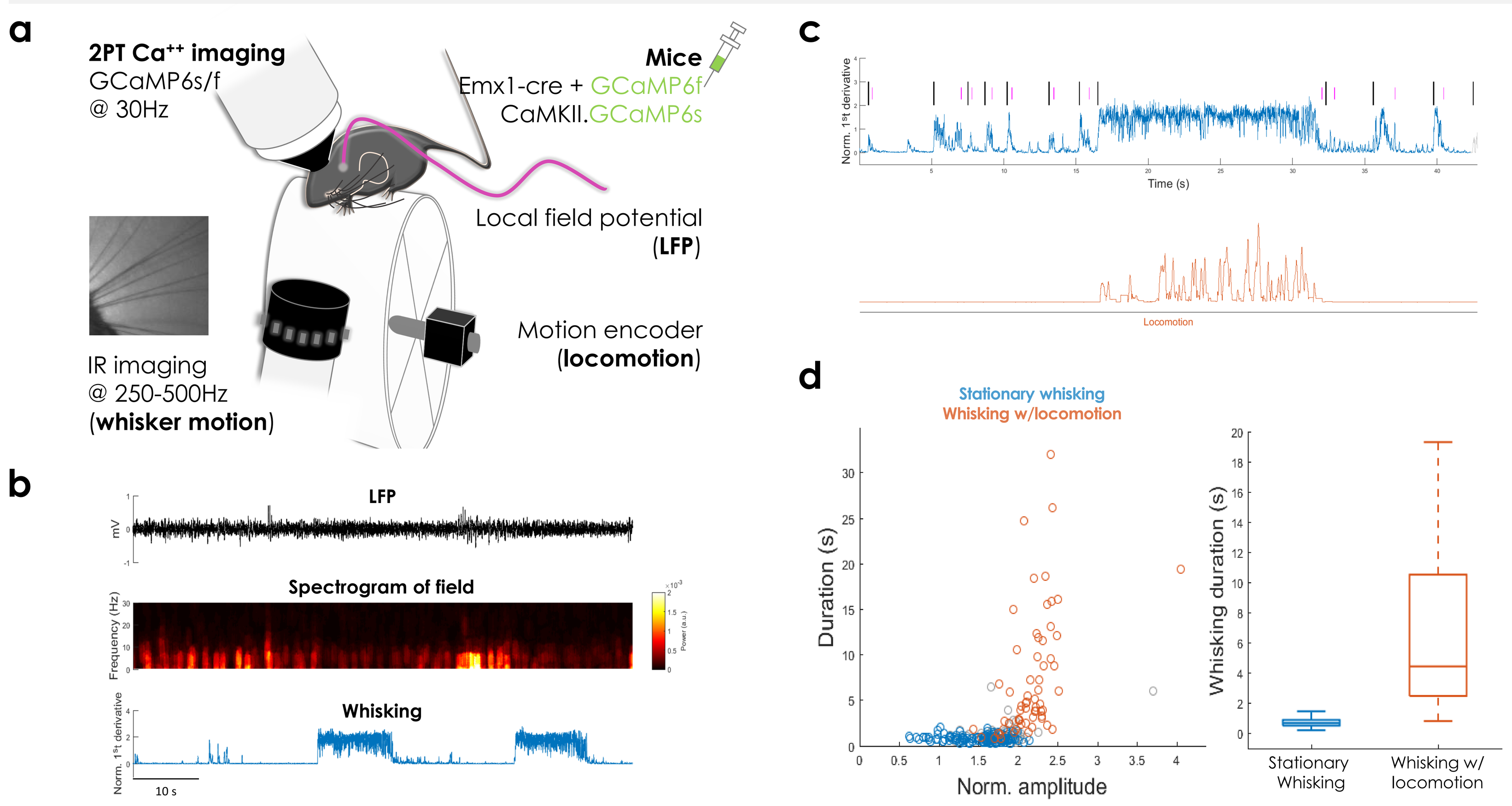
INTRODUCTION

Superficial cortical layers, also known as associative layers, are the main gateway for corticocortical communication. Principal neurons in superficial layers of sensory cortices exhibit highly heterogeneous patterns of activity, not only in response to external stimuli, but also in relation to different aspects of an animal's behavior. This observation indicates the presence of functionally organized subnetworks within associative layers. To understand the structural organization of these cortical subnetworks, we focused on a functionally-defined subpopulation of neurons in primary somatosensory cortex (S1bf) that is highly sensitive to behavioral state (exploratory behavioral state). We aimed to: **1)** identify a subpopulation of neurons in superficial layers of S1bf that is highly sensitive to exploratory behavior, **2)** evaluate the stability of neuronal activity of this subpopulation over multiple days, and **3)** analyze the presynaptic ensembles of the functionally identified subpopulation, anatomically and functionally.

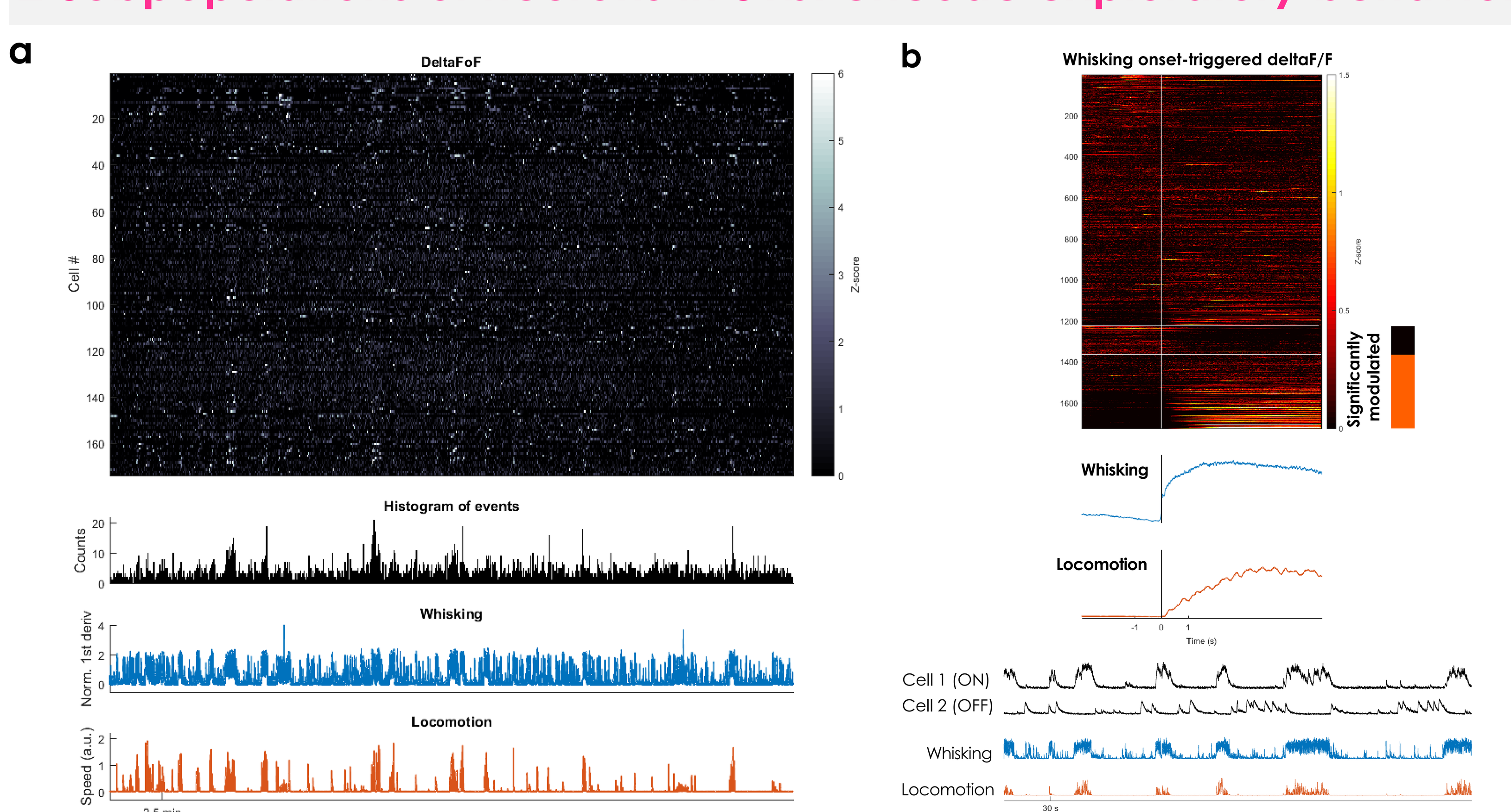


1 Exploratory behavior



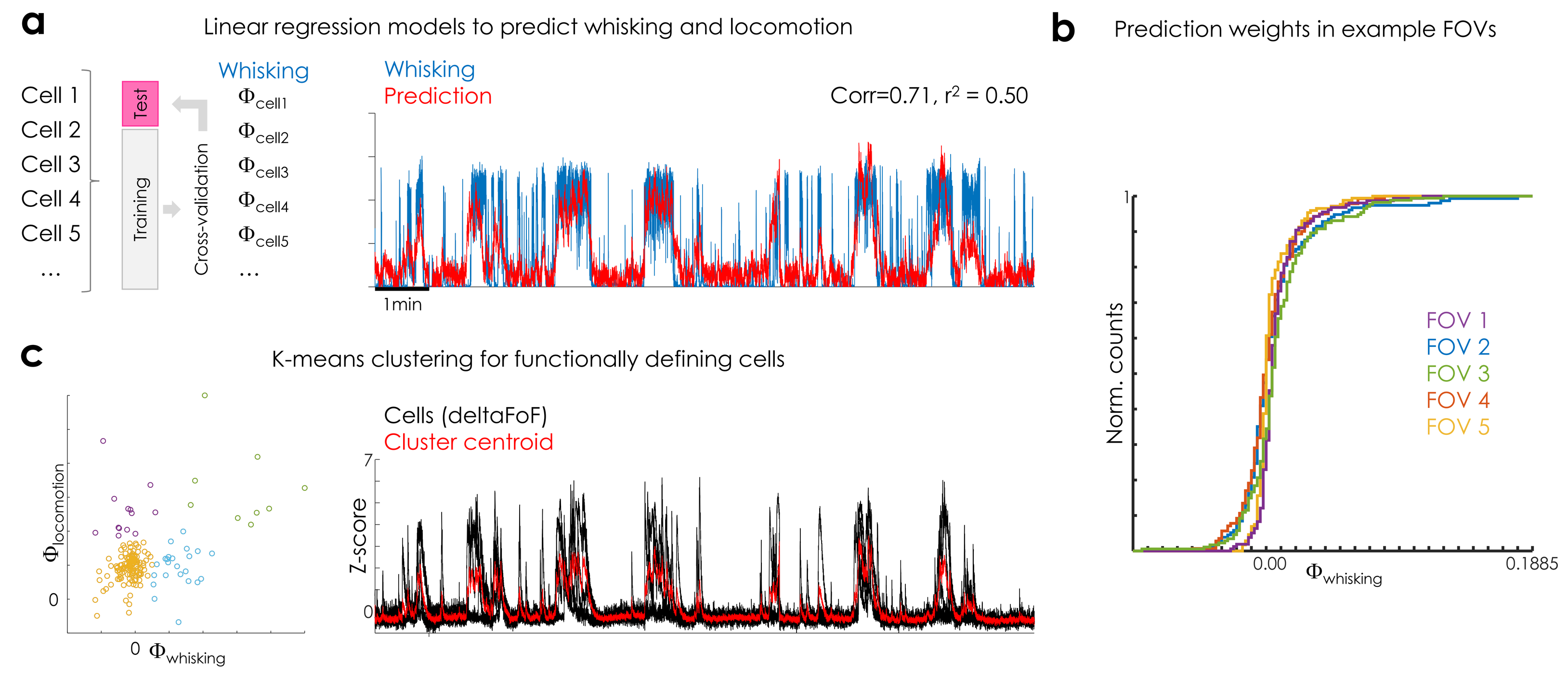
Exploratory behavior. a) Experimental setup. **b)** Example LFP trace denoting instantaneous changes in frequency components during behavioral transitions. **c)** Detection of whisking and locomotion events. **d)** Distribution of whisking event duration for one recording session; whisking events are color-coded based on the co-expression of locomotion (blue: stationary; orange: locomotion). Right panel, p<0.05 (Mann-Whitney).

2 Subpopulations of neurons in S1bf encode exploratory behavior



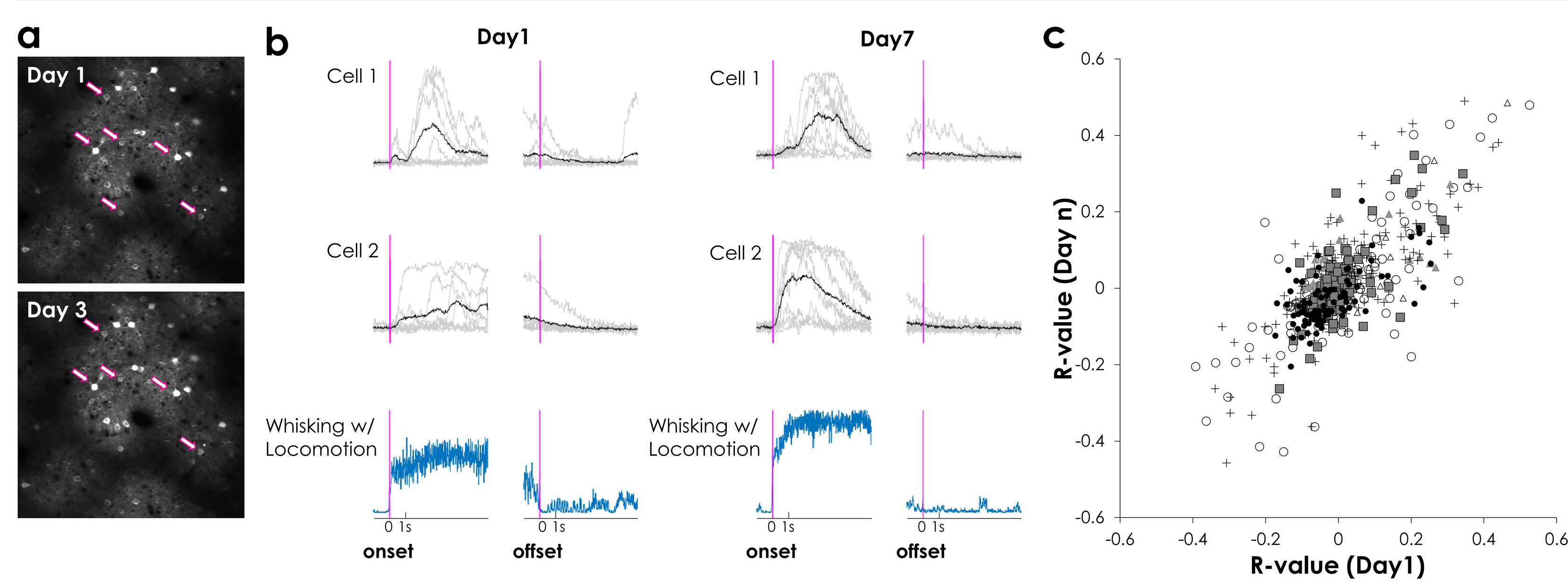
Heterogeneous neuronal activity during exploratory behavior. a) DeltaF/F and behavioral traces over a recording session. **b)** Whisking-locomotion onset-triggered average z-scored deltaF/F (1724 cells, 151±30 cells per field of view, FOV, n=4 mice); significantly modulated cells are indicated by the vertical bar (black: downregulated; orange: upregulated, p<0.05, paired t-test).

3 Neuronal activity from S1bf can predict exploratory behavior



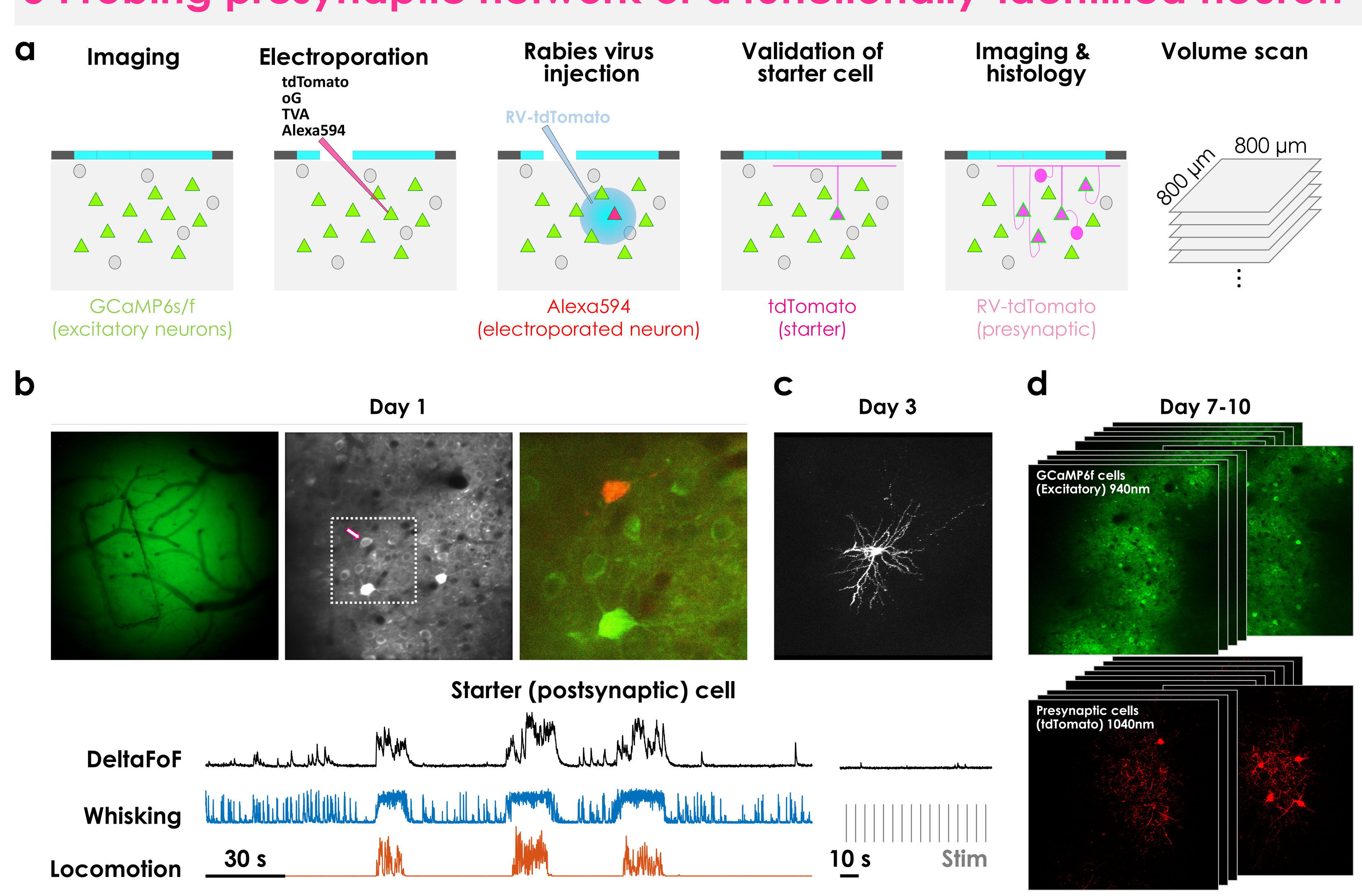
Prediction of exploratory behavior from neuronal activity using linear regression. a) Prediction of whisking and locomotion using linear regression models. **b)** Normalized cumulative distribution of whisking-prediction weights for all neurons per FOV (n = 3 animals). **c)** Whisking-prediction weights as a function of the locomotion-prediction weights for individual neurons; clustering of neurons using k-means (clusters are color coded).

4 Correlation of neural activity with behavior is highly stable



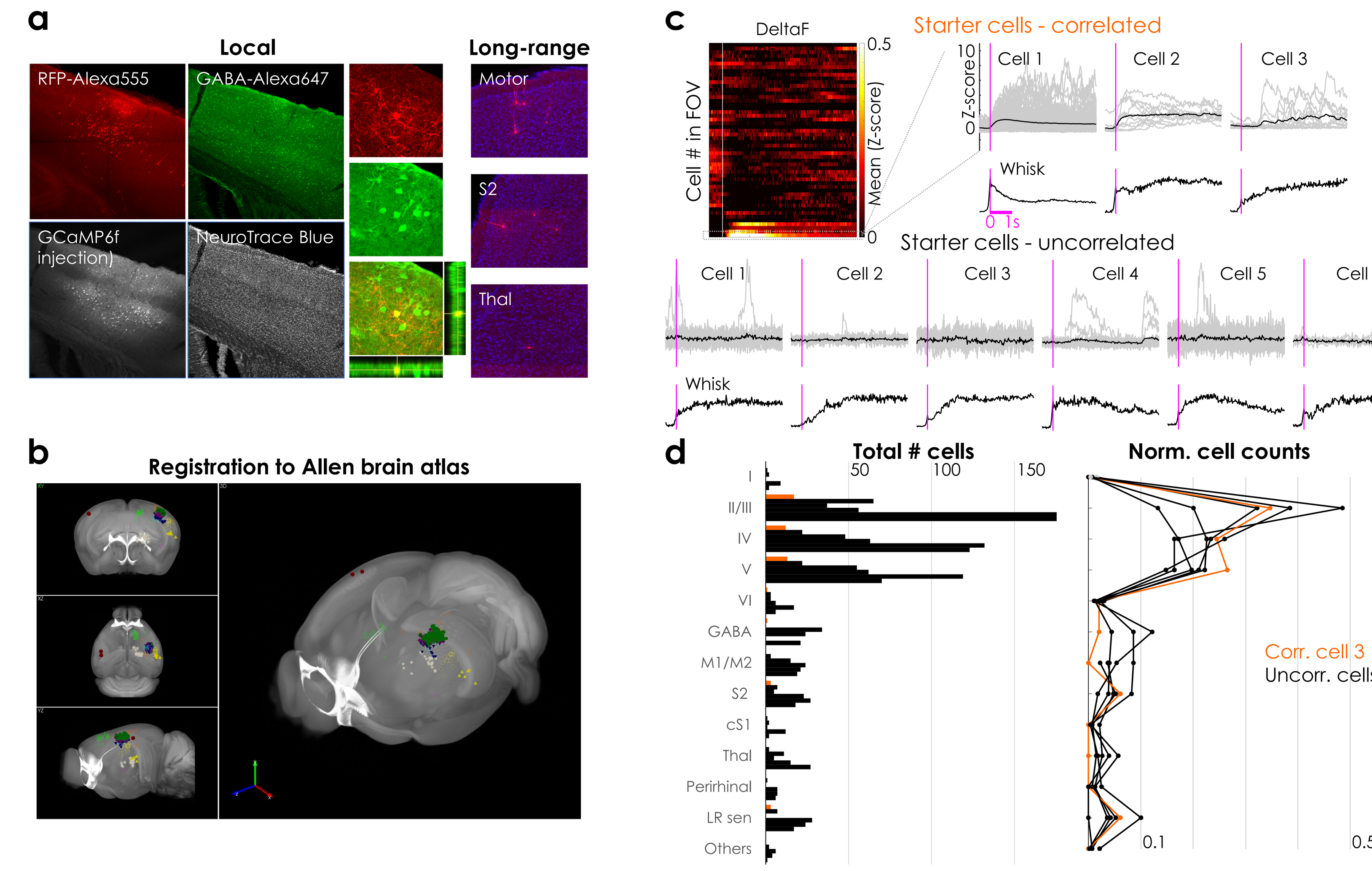
Stability of the correlation between spontaneous neuronal activity and exploratory behavior across days. a) 2PT Ca++ imaging FOV for two recording sessions. **b)** Whisking-locomotion onset- and offset-triggered deltaF/F for two example cells recorded across different days. Black: mean trace. Grey: individual traces. Blue: mean whisking trace. **d)** Individual neurons whisking Pearson's correlation coefficient for imaging session *n* as a function of the whisking Pearson's correlation coefficient computed for an earlier imaging session. Different markers indicate different FOVs (n = 6 FOVs, 5 animals).

5 Probing presynaptic network of a functionally-identified neuron



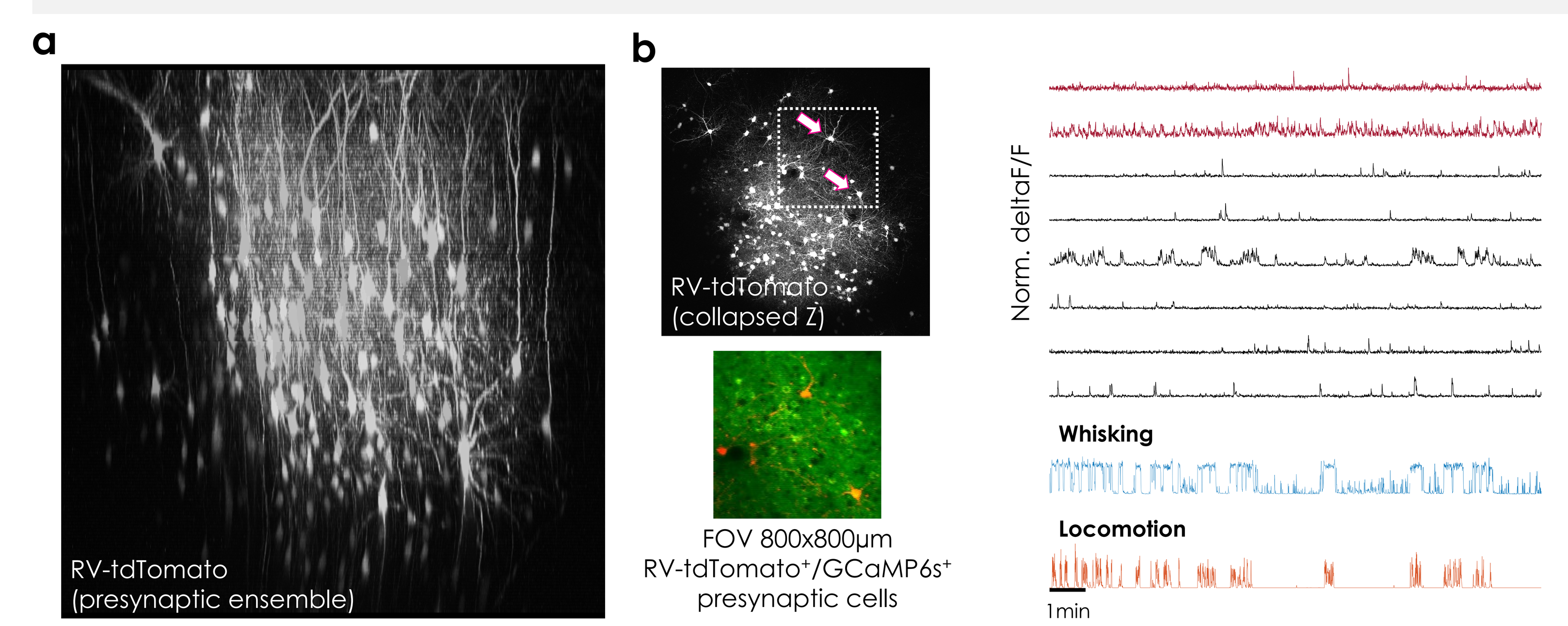
Monosynaptic retrograde tracing from a single behaviorally-defined excitatory neuron. a) Experimental outline. **b)** Electroporation of an example exploratory behavior-predicting neuron. Left: cranial window with a custom-made opening. Middle: 2PT Ca++ imaging FOV; arrow denotes the neuron selected for electroporation. Right: neuron filled with Alexa594 upon electroporation. Lower: selected neuron behavioral tuning and (lack of) response to whisker stimulation. **c)** Neuron expressing tdTomato 3 days after electroporation. **d)** Volume imaging.

6 Anatomical organization of presynaptic ensembles



Anatomical organization of presynaptic ensembles of functionally identified L2/3 pyramidal neurons. a) Brain-wide red fluorescent protein (RFP), GABA and NeutroTrace Blue (immuno)histochemistry and 4-channel confocal (local) and epifluorescence (long-range, LR) images. **b)** Semi-automated RFP+ cell segmentation, registration to Allen brain atlas and spatial clustering. **c)** Behavioral tuning of nine starter cells. DeltaFoF aligned to the onset of whisking events (time 0): gray - individual traces; black - mean. **d)** Brain-wide distribution of presynaptic neurons for five starters cells (as in **c**).

7 Functional properties of presynaptic ensembles



Functional organization of local presynaptic neurons. a) Structural 2PT Ca++ imaging: 3D-reconstruction images acquired from 0-800 µm of depth from dura for an example animal. **b)** Activity of example presynaptic excitatory neurons (tdTomato+/GCaMP+), in red, *versus* non-presynaptic local excitatory neurons (tdTomato-/GCaMP+).

SUMMARY

- Structure within spontaneous patterns of activity can be uncovered, at least in part, by quantifiable motor activity
- Neuronal patterns of activity in relation to exploratory behavior are heterogenous
- Exploratory behavior can be predicted from neuronal activity
- A minority of neurons is responsible for most of the prediction accuracy (robust)
- Neuronal behavioral tuning is maintained over days and weeks, at the single neuron level
- Sensory responsiveness is not skewed towards a particular behavioral tuning
- We analyzed presynaptic ensembles of single neurons of orthogonal behavioral tunings, in terms of excitatory cell firing patterns and whole brain excitatory and inhibitory spatial architecture
- With an increased number of analyzed presynaptic ensembles, we will conclude if connectivity principles rule the heterogenous representation of behavior in S1bf

ACKLOWDGEMENTS

Jia Qi, Shovan Naskar, Jonathan Krynitsky, Ron Paletzki, NIMH Section on Instrumentation, NIMH Behavioral Core, CNNV

FUNDING

NIH Intramural Research Program