**Supplementary Materials for**

# Large-scale analysis reveals populational contributions of cortical spike rate and synchrony to behavioural functions

Rie Kimura[1,2,3,4], Akiko Saiki[1,2], Yoko Fujiwara-Tsukamoto[1,2,5], Yutaka Sakai[1,2] and Yoshikazu Isomura[1,2]

The Journal of Physiology, 2016.

[1]*Brain Science Institute, Tamagawa University, Tokyo, Japan*

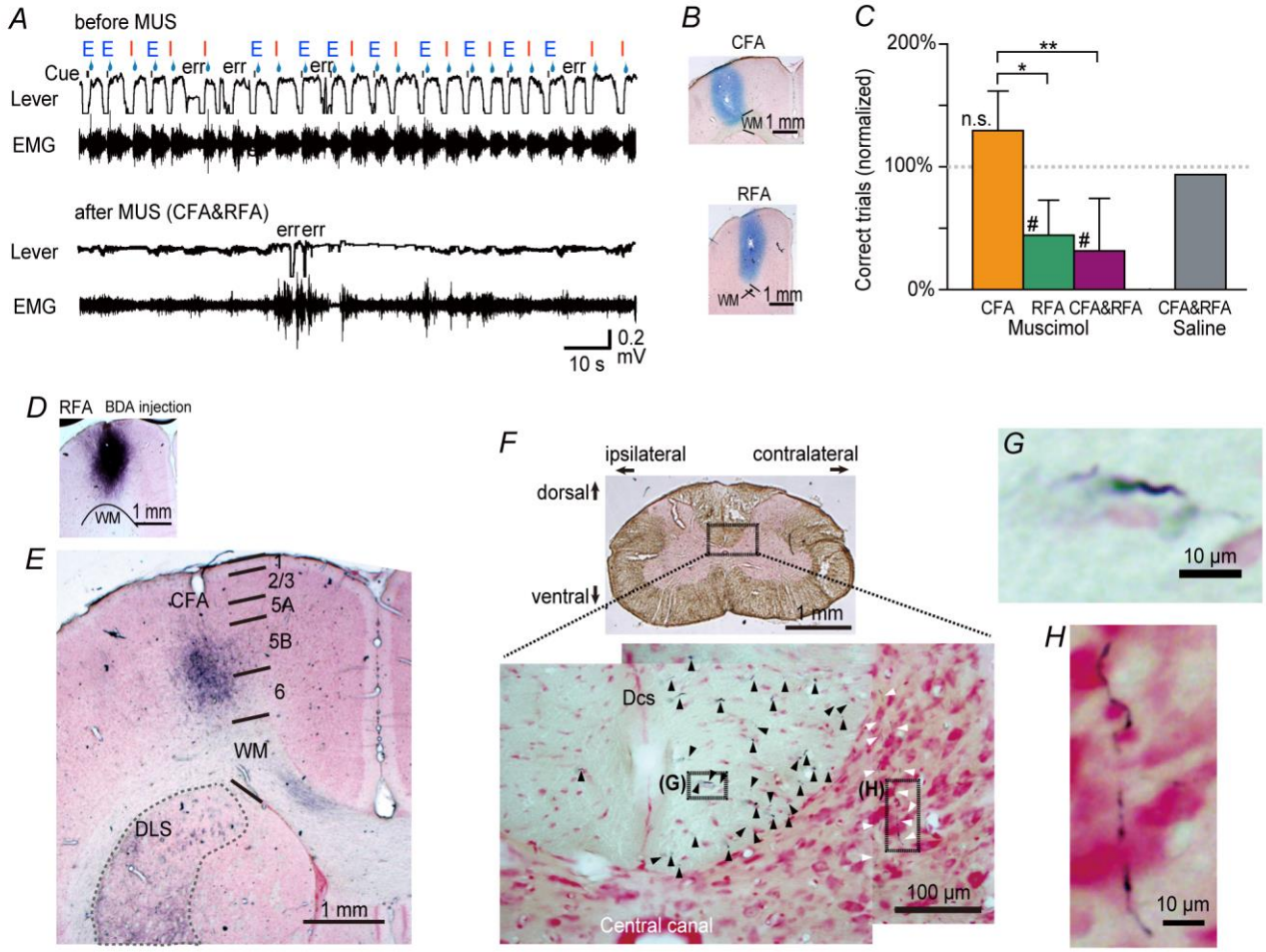
[2]*JST CREST, Tokyo, Japan*

[3]*Division of Visual Information Processing, National Institute for Physiological Sciences, National Institutes for Natural Sciences, Okazaki, Japan*

[4]*Department of Physiological Sciences, SOKENDAI (The Graduate University for Advanced Studies), Okazaki, Japan*

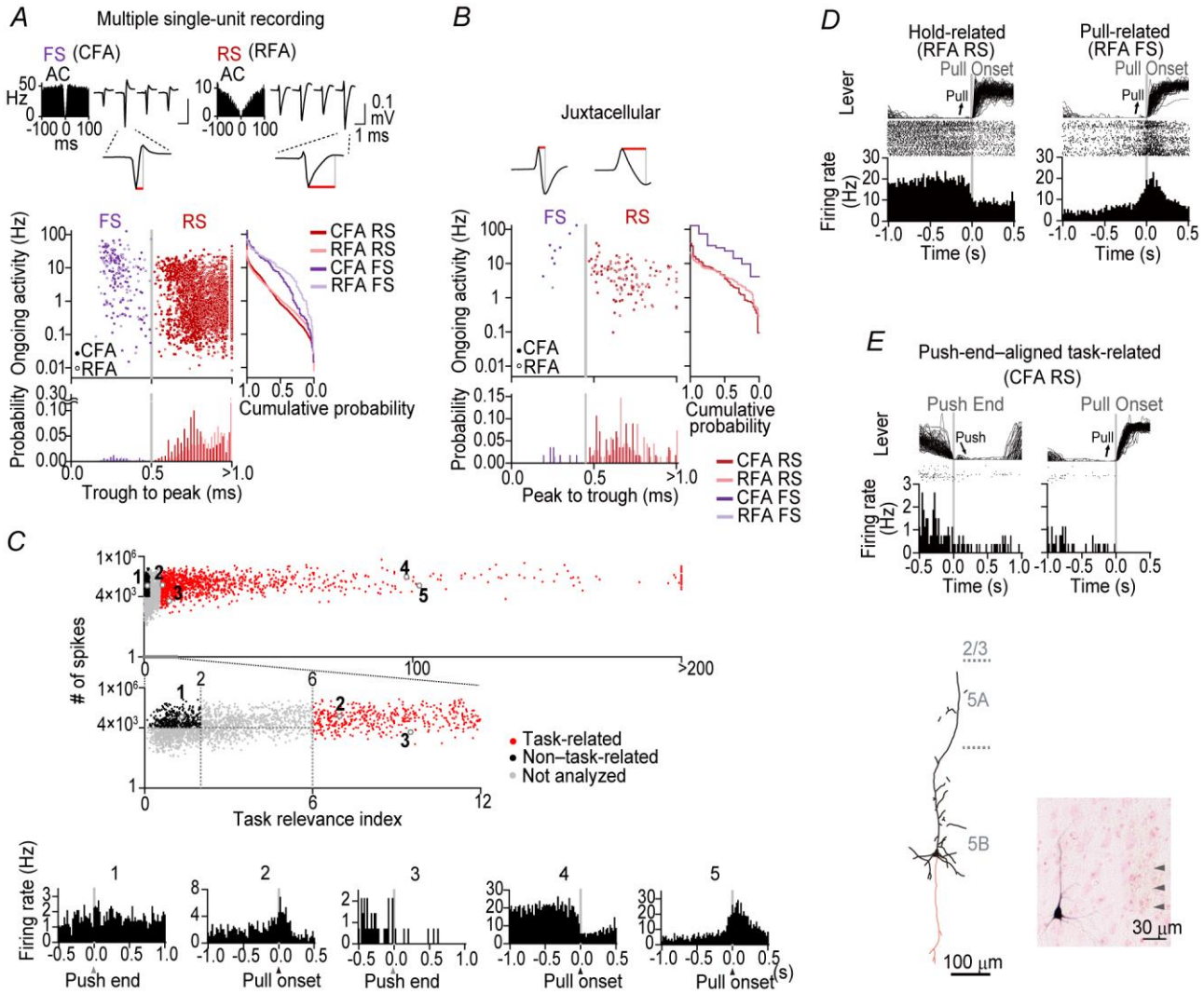
[5]*Laboratory of Neural Circuitry, Graduate School of Brain Science, Doshisha University, Kyoto, Japan*

## Supplementary Material 1. Task performance depending on motor cortices




*A*, representative lever trajectories and EMG activities before and after unilateral inactivation of both CFA and RFA by muscimol injection (MUS, 1 µg/µl, 0.5 µl). *B*, estimations of the spread of muscimol injection by injection of Pontamine sky blue dye in preliminary experiments. *C*, averaged change in task performance consisting of both external- and internal-trigger trials 15–30 min after unilateral CFA/RFA inactivation by muscimol. There was a significant effect of muscimol on task performance ($p < 0.01$, one-way ANOVA). Task performance is defined as the number of correct trials, normalized against trials performed 5–15 min before injections (CFA MUS, 129 ± 32 %, $N = 6$, n.s., $p = 0.07$; one-sample *t*-test compared with 100%; RFA MUS, 44.2 ± 28.5%, $N = 4$, #, $p = 0.02$; CFA&RFA MUS, 31.4 ± 42.6%, $N = 4$, #, $p = 0.04$; CFA&RFA Saline, 93.6%, $N = 2$, n.s., $p = 0.8$; **, $p < 0.01$, *, $p < 0.05$ by Scheffé's *F* test). *D*, BDA injection site in RFA. *E*, anterograde labeling in ipsilateral CFA and dorsolateral striatum (DLS; dotted area) by injection of BDA into RFA (*D*). *F*, anterograde labeling (arrowheads) in contralateral spinal cord by the same injection of BDA. Dcs, dorsal corticospinal tract. *G*, enlarged view of labeled corticospinal axon perpendicular to the section in (*F*), bottom. *H*, another axon horizontal to the section.

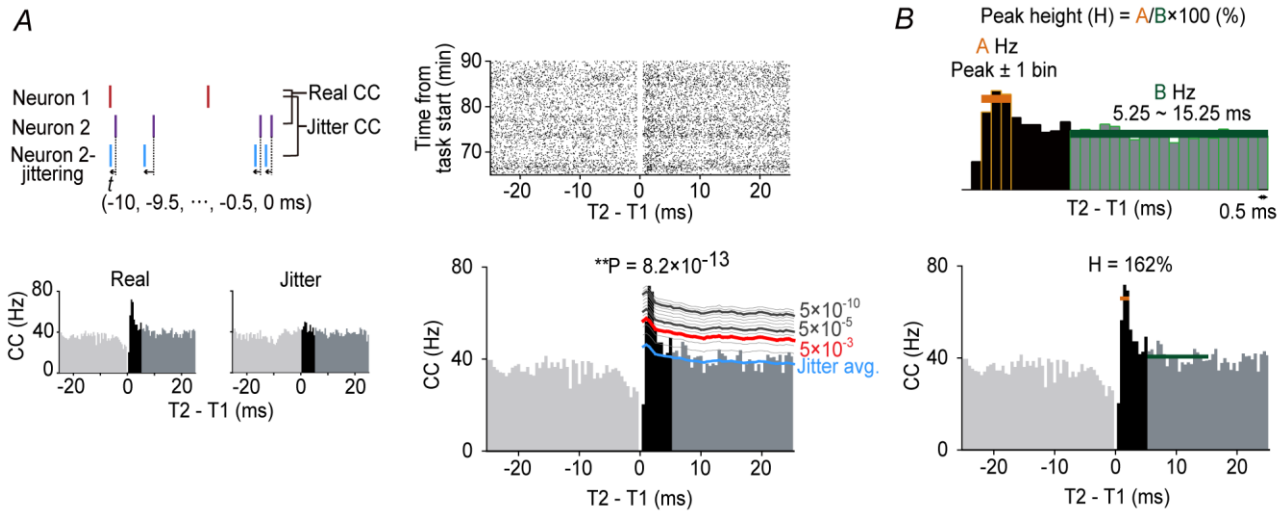
## Supplementary Material 2. Electrophysiological analyses of spike activities




*A*, classification of neurons (RS and FS neurons) in multiple single-unit recording. Top, examples of auto-correlogram (AC) and averaged spike waveforms. Bottom, relationship between ongoing spike rate and spike duration from trough to peak for each neuron (upper left), cumulative histograms of ongoing spike rate (right), and histograms of spike duration (lower). Neurons were classified into FS and RS based on the spike duration threshold (gray vertical line). *B*, classification of juxtacellularly recorded neurons as in (*A*). *C*, classification of neurons into task-related (red) and non–task-related (black) neurons. Task relevance index was defined as $-\log_{10} P$ using the significance *p*-value (P) of the KS test for differences between the uniform distribution and the peri-event time histograms (PETHs) aligned to push-end/pull-onset in external-/internal-trigger trials (see Methods). Task-related and non–task-related neurons were defined using the task relevance index and the total number of recorded spikes. Bottom, representative examples of PETHs. Numbers correspond to the values in the upper graphs. 1: non–task-related neurons; 2–5: task-related neurons. 1,

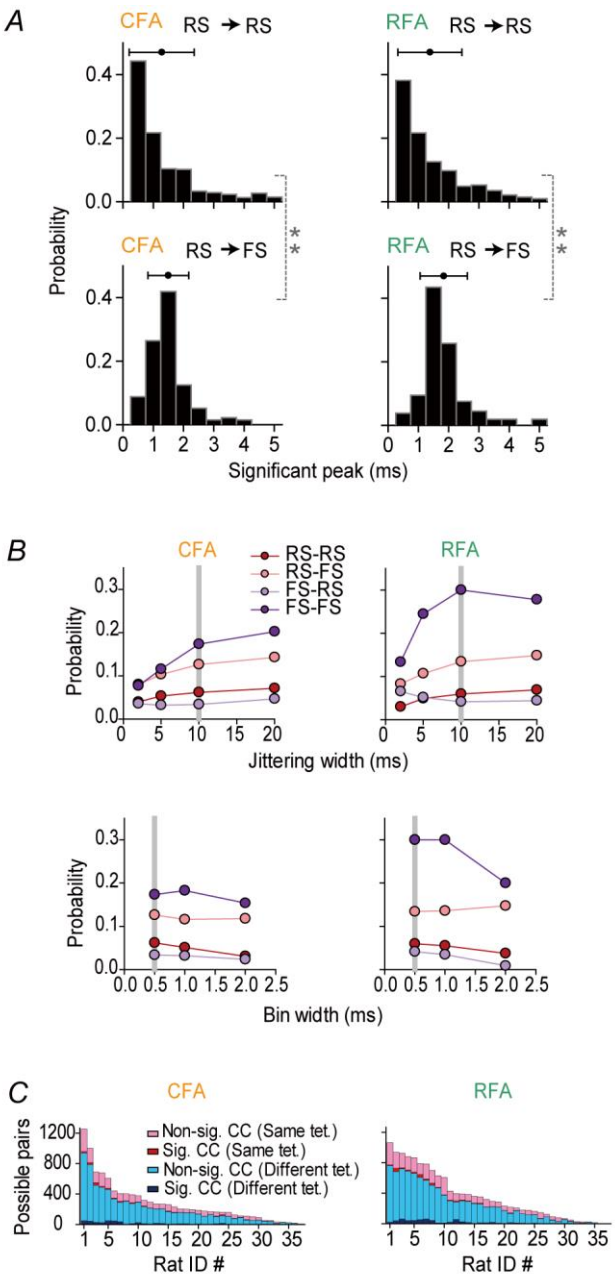
2, 4, 5: multiple single-unit recording; 3: juxtacellular recording. *D*, two representative neurons exhibiting distinct patterns of spike activity in relation to pull movement (top, lever trajectories; middle, raster plots; bottom, PETH of spike activities aligned to pull-onset in both external- and internal-trigger trials). *E*, representative layer 5B pyramidal neuron identified by the juxtacellular method. Upper: top, lever trajectories; middle, raster plots; bottom, PETHs aligned to push-end or pull-onset. Lower: reconstructed morphology of the recorded neuron (black, soma and dendrites; red, axons; scale bar, 100 μm). Inset, ABC staining of the juxtacellularly recorded neuron, and electrode tracks (gray arrowheads) for multiple single-unit recording. Scale bar, 30 μm.

## Supplementary Material 3. Evaluation of spike synchrony



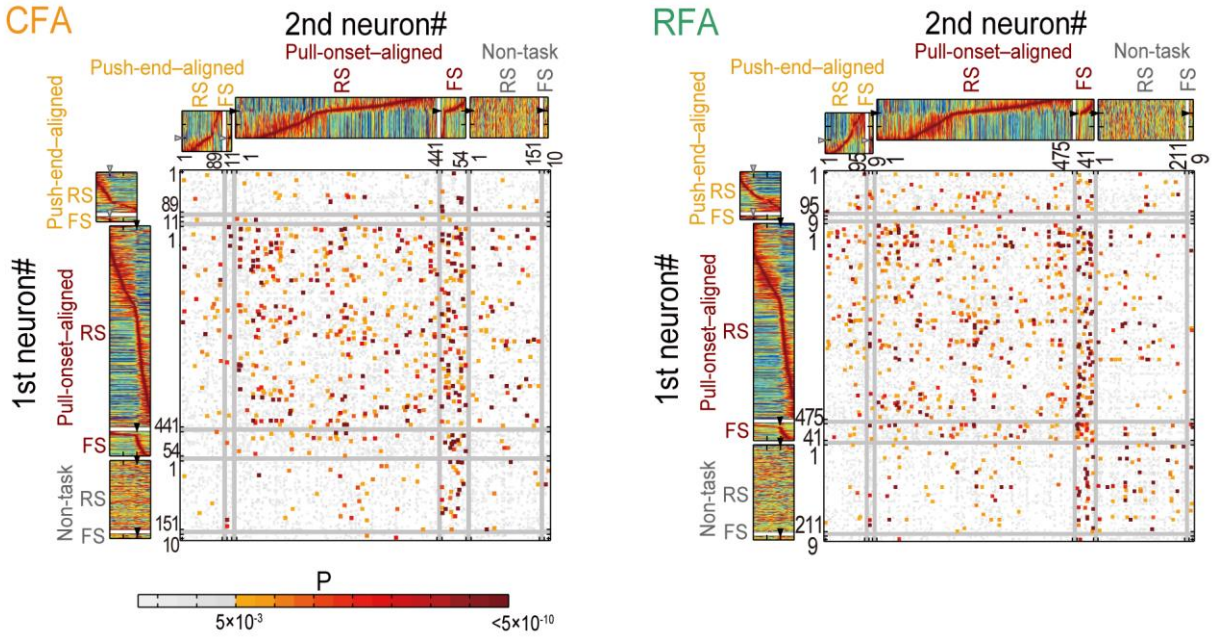

*A*, evaluation of statistical reliability of spike synchrony. Top left: for a real directional pair (neuron 1 to neuron 2), each spike timing of neuron 2 was jittered uni-directionally by a step randomly sampled from –10, –9.5, …, ⁻0.5, 0 ms. Bottom left: the real CC and a sample of jitter CCs for an example neuron pair. We focused on peaks within 5 ms (10 bins, black regions). Top right: temporal stability of spike timings between the pair of neurons. Bottom right: the probability distribution of jitter CCs could be estimated by binomial distributions (see Methods). The probability (*P*) that the peak within the 10 possible bins for a jitter CC is larger than actually observed peak represents the statistical significance of the actually observed peak. The contours of *P*-value were plotted. *B*, evaluation of strength of spike synchrony. The relative peak height (*H*) of a CC (bin width; 0.5 ms) is defined as the ratio of the mean CC at the peak bin and adjacent bin(s) (two bins for a peak in the 0.5-ms bin, and three bins otherwise; orange horizontal bar) to the mean CC within the basal range 5.25–15.25 ms (20 bins; green horizontal bar).

## Supplementary Material 4. The validity of evaluation methods of significant spike synchrony




*A*, histograms of spike delays of significant peaks ($P < 0.005$) in CCs, along with means and SD (above histograms; black circles and error bars). These differed between subtype combinations (** marked by KS test). *B*, ratio of neuron pairs with significant spike synchrony ($P < 0.005$), which was robust with respect to parameter changes (top: jittering width, bottom: bin width of CC). Hereafter, we adopted 10 ms for jittering width and 0.5 ms for bin width (gray vertical lines). *C*, ratios of neuron pairs with significant spike synchrony in the same/different tetrode across rats (CFA: $N = 37$, RFA: $N = 36$).

**Supplementary Material 5. Significant spike synchrony and behavioral movement functions**




Relationship between significant spike synchrony and the pull/push-related activities in CFA or RFA. The *P*-values of significant CC peaks ($P < 0.005$) are plotted as pseudo-colored dots on the combination of neurons sorted by the peak time of PETHs (in the same order as in Figure 2*B*). Those with non-significant peaks ($P \geq 0.005$) are plotted in gray. PETH of each neuron is presented for either external- or internal-trigger trials (with a larger peak for a task-related neuron, or with larger task-relevance index for a non–task-related neuron).