

## SUPPLEMENTAL RESULTS & DISCUSSION

### *Extension of Tetrode Recording and T-maze Procedural Task to Mice*

This study extended directly previous experiments in which we trained rats to perform similar T-maze procedural tasks and recorded ensemble neuronal activity in the dorsolateral striatum during the course of training (Barnes et al. 2005; Jog et al. 1999). Here, in order to perform comparable analyses in mice, we developed light-weight headstages and amplifiers for recordings with 4 tetrodes (instead of up to 24 in rats), and modified the T-maze (smaller size, alleyways instead of elevated tracks). The results of the study were remarkably similar to those obtained previously in rats, except for behavioral and neuronal differences at the task start as described below. The mice acquired the task at rates equivalent to that of the rats, and they performed the task consistently once it was acquired. The ensemble activity patterns that developed as the mice learned the task were nearly comparable to those found in previous studies. The quality of neuronal recording, particularly high stability of day-to-day recording, was as good as, if not better than, that in rats. The present study thus demonstrates convincingly the feasibility of performing long-lasting chronic large-volume recordings with multiple tetrodes in freely-moving mice actively engaged in behavioral tasks, and suggests high promise for behavioral-electrophysiological work with genetically modified mice.

### *Variability in Reaction Time to Gate Opening*

The latency of onset of locomotion following the opening of the start gate decreased during learning of the auditory and tactile versions of the T-maze task, but this measure was highly variable from trial to trial and from mouse to mouse. This large variability in reaction times to gate opening in the mice stands in contrast to the consistently rapid locomotion onset of

rats in similar T-maze tasks, whether or not there was an initial warning cue before gate opening (Barnes et al. 2005; Jog et al. 1999). These results suggest that the warning cue and the opening of the gate were less salient for mice than for rats as stimuli indicating the start of trials.

#### *Emergence of MS Ensemble Activity to Gate Opening late in Auditory Training*

Robust responses time-locked to the opening of the start gate were found in the average ensemble activity of task-related MS units during early stages of training on the tactile version (Fig. 3A and Supplemental Figs. S2, S3 and S5), suggesting a possibility that the switch in modalities of conditional cues induced this activity. However, analysis of 10 daily sessions around the cue switch indicated that this activity was present both before and after the switch (Figs. 4A and 5A), and it is likely that the gate-related discharges developed as the mice were overtrained on the task. In the comparable study with rats, similar robust responses around click warning cue and gate opening developed earlier in training (Barnes et al. 2005). It is possible that this development was delayed in mice because of low saliency of start cues for the mice, as indicated by behavioral measures described above.

The development of responses at gate opening late in overtraining on the auditory version was not apparent when average activity was plotted for learning stages, because the last auditory sessions for individual mice belonged to different stages. Plots for daily session averages (Supplemental Fig. S5) did not show the gate-related discharges either, because the sample sizes were too small toward the end of auditory training.

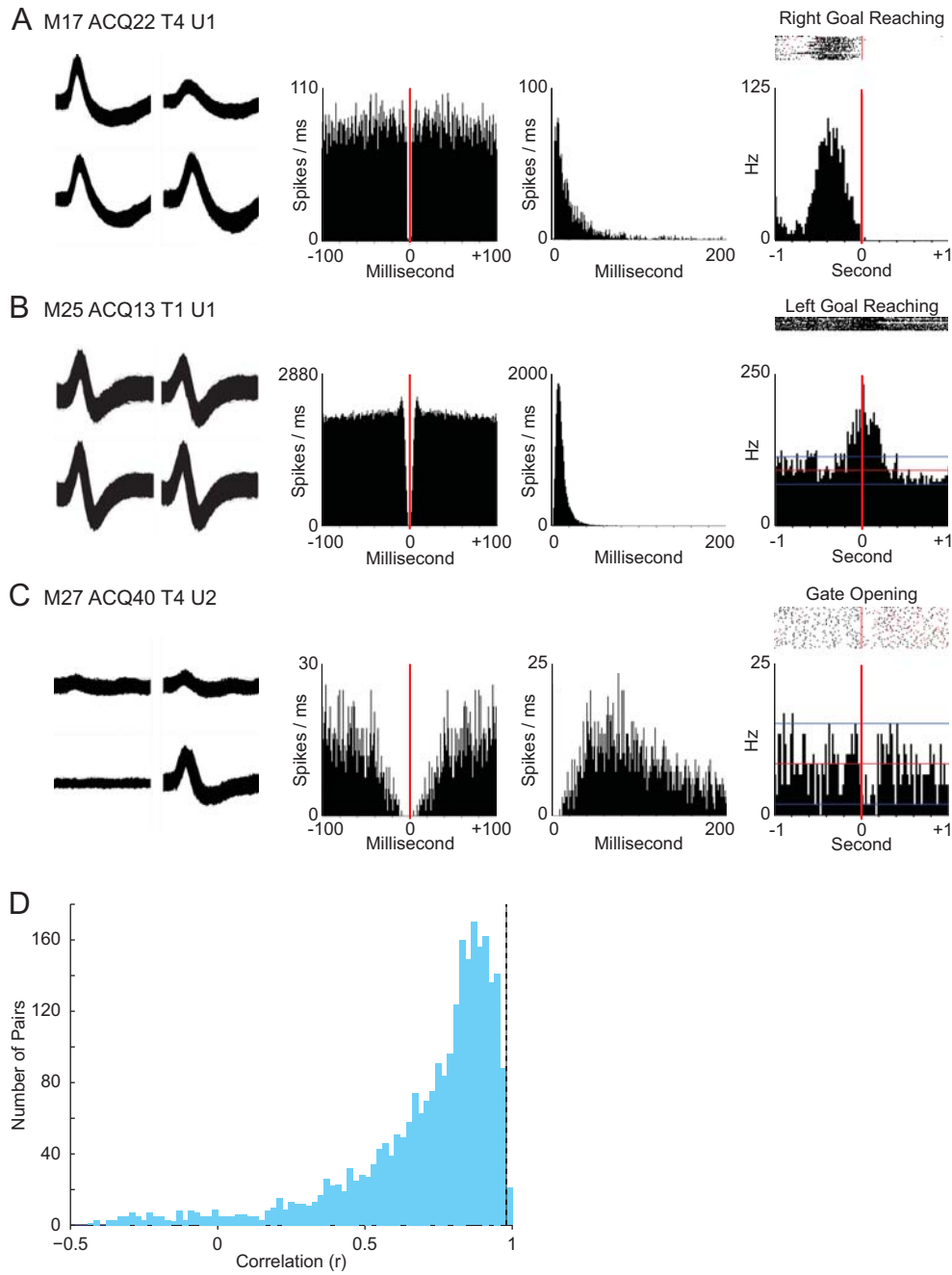
#### *Tracking of Putative Single Neurons across Multiple Sessions*

In order to test whether single neurons adjust task-related responses when a change in task condition is introduced, we identified PSNs that were recorded over daily sessions by using a spike-waveform correlation method originally reported by Emondi et al. (2004). We tested for significant changes in responses across the cue switch.. We used this method because our spike data were often remarkably stable over many daily sessions. We are aware that we still may make false positive errors by matching two different units incorrectly. To minimize this possibility, we performed 1000 comparisons by shuffling our dataset and, based on these empirical data, set the threshold (correlation greater than 0.98) so that the likelihood of error was less than 0.01. The methodology of single-cell tracking in behaving animals that we used here, as well as those developed by others (Tolias et al. 2007), will undoubtedly provide critical information about single-cell plasticity that occurs during slow acquisition of behavioral procedures and habits.

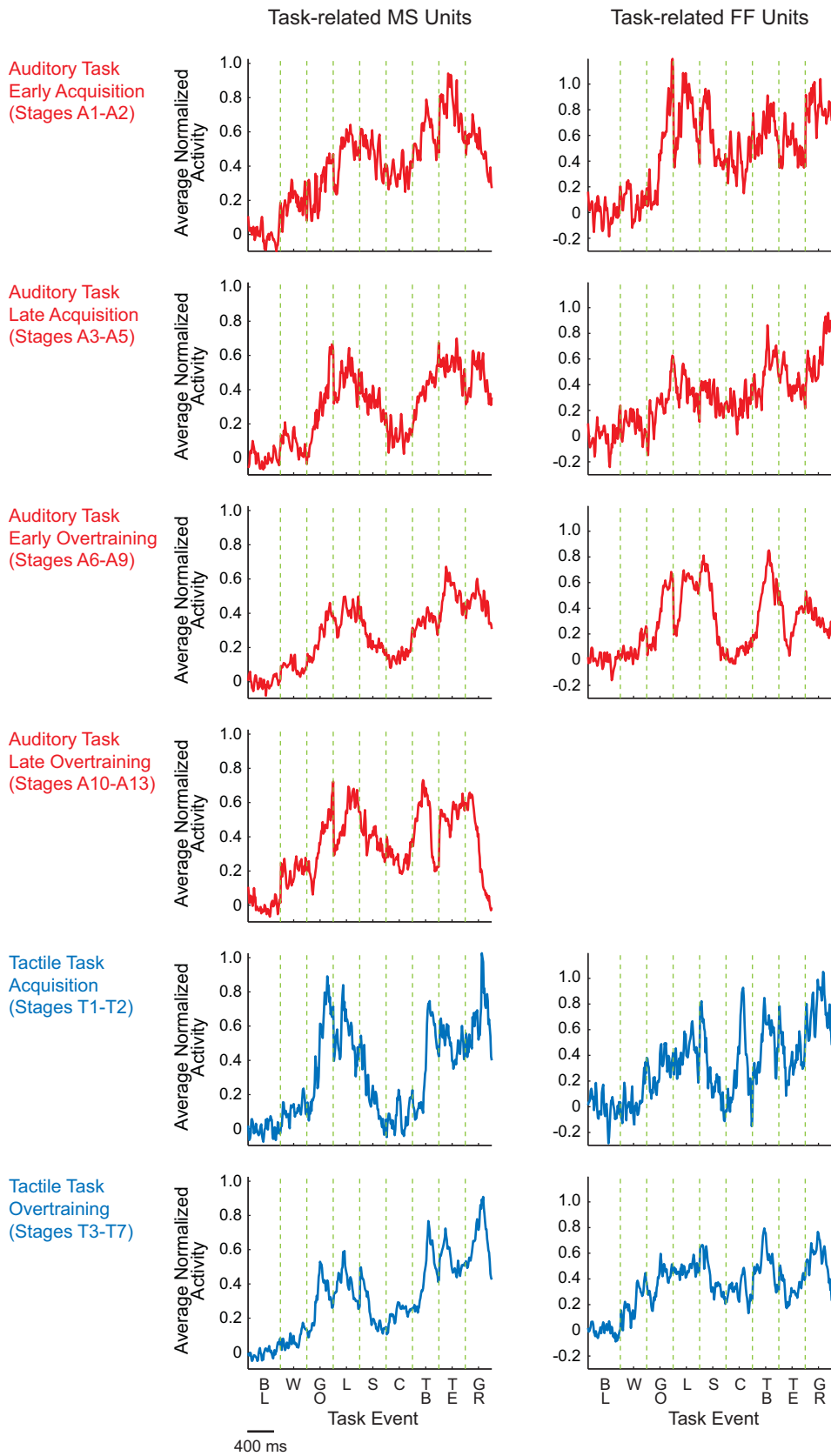
## REFERENCES

- Barnes T, Kubota Y, Hu D, Jin DZ, and Graybiel AM.** Activity of striatal neurons reflects dynamic encoding and recoding of procedural memories. *Nature* 437: 1158-1161, 2005.
- Emondi AA, Rebrik SP, Kurgansky AV, and Miller KD.** Tracking neurons recorded from tetrodes across time. *J Neurosci Methods* 135: 95-105, 2004.
- Jog M, Kubota Y, Connolly CI, Hillegaart V, and Graybiel AM.** Building neural representations of habits. *Science* 286: 1745-1749, 1999.

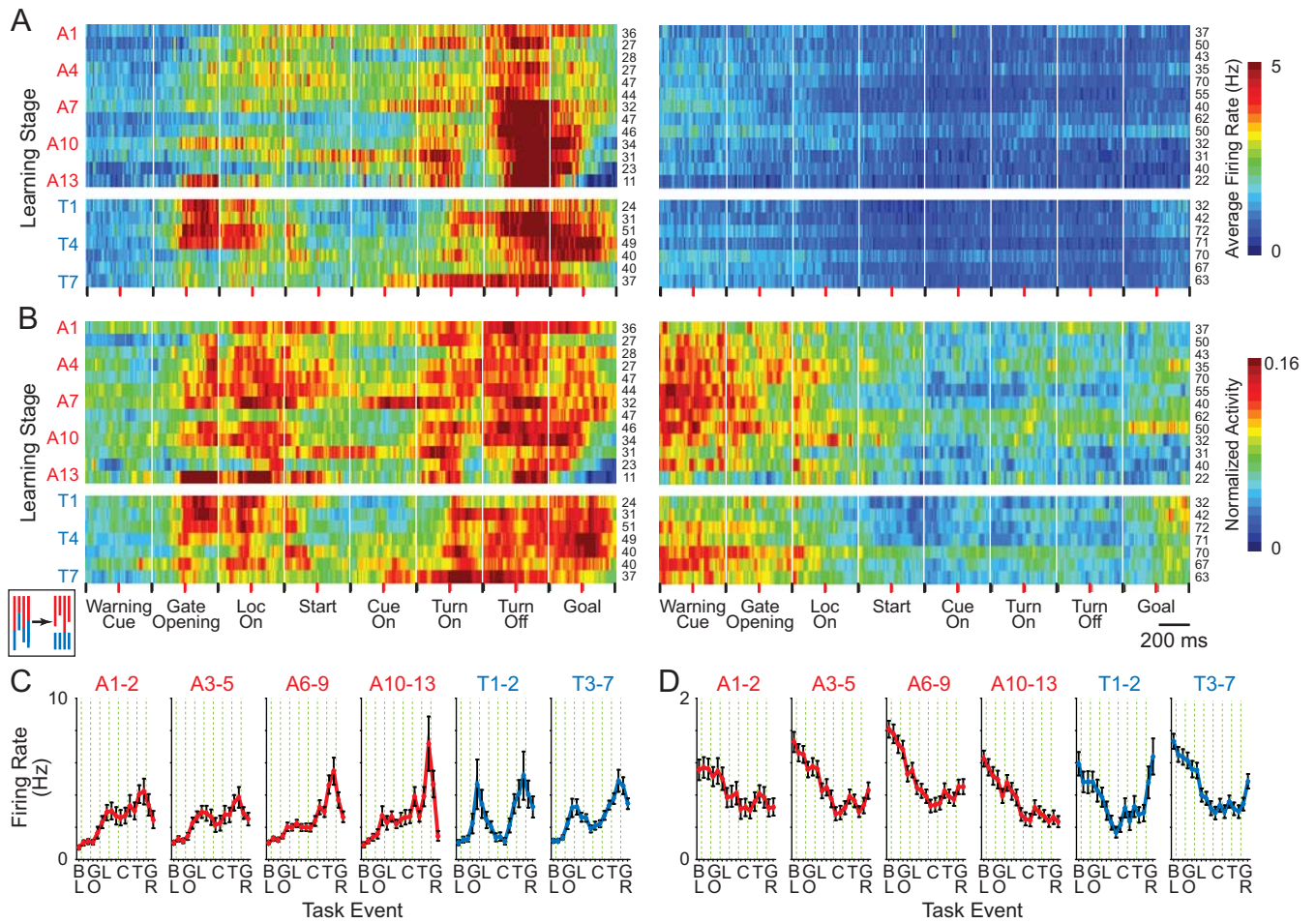
**Tolias AS, Ecker AS, Siapas AG, Hoenselaar A, Keliris GA, and Logothetis NK.** Recording chronically from the same neurons in awake, behaving primates. *J Neurophysiol* 98: 3780-3790, 2007.



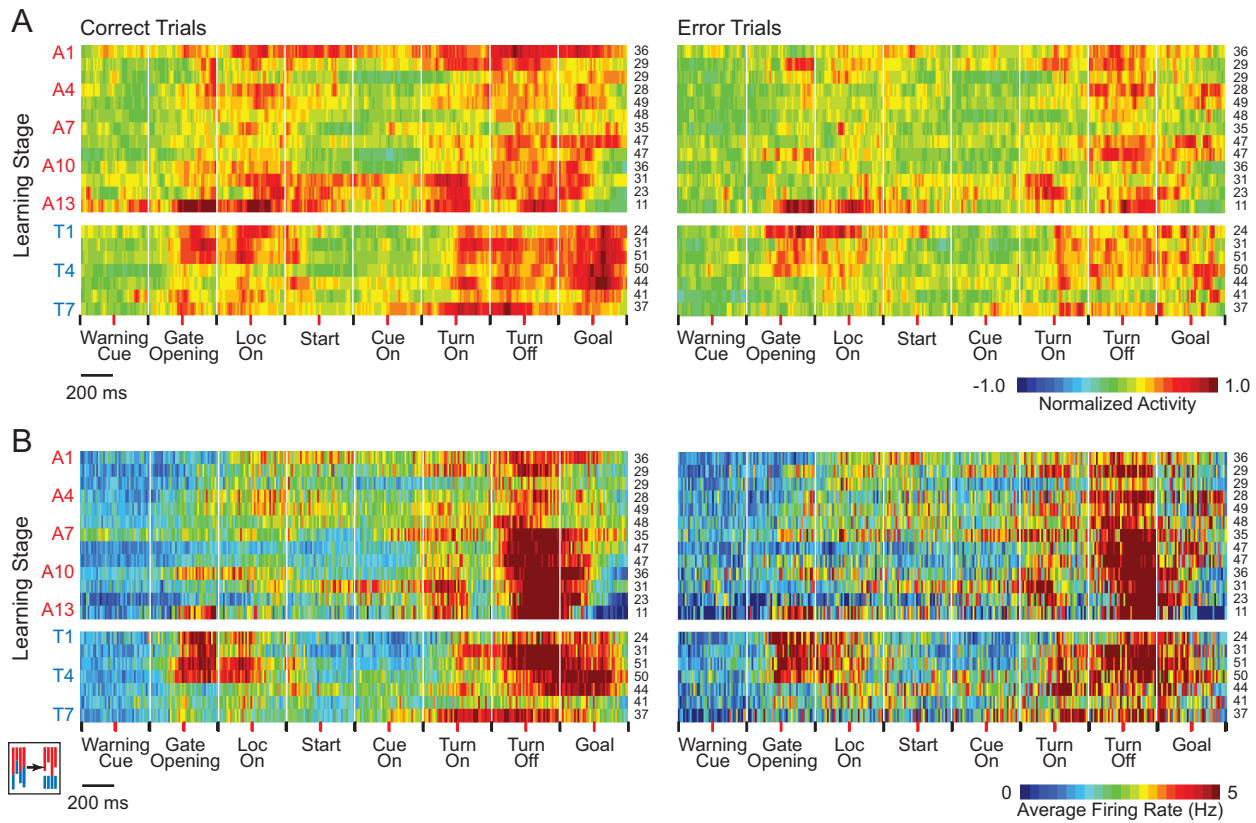
Supplemental Figure 1. Methods for neuronal data analyses. *A-C*: Examples of putative MS (*A*), FF (*B*) and TAN (*C*) units with spike waveforms, autocorrelograms, interspike interval plots and peri-event histograms (left to right). *D*: Histogram illustrating the distribution of spike waveform correlations for shuffled data. Correlation of  $>0.98$  (dashed line,  $P < 0.01$ ) was used as a criterion for putative single units based on this result.



Supplemental Figure 2. Average normalized activity of task-related MS (*left*) and FF (*right*) units during 6 phases of training on the auditory (red) and tactile (blue) versions of the T-maze task (as labeled).

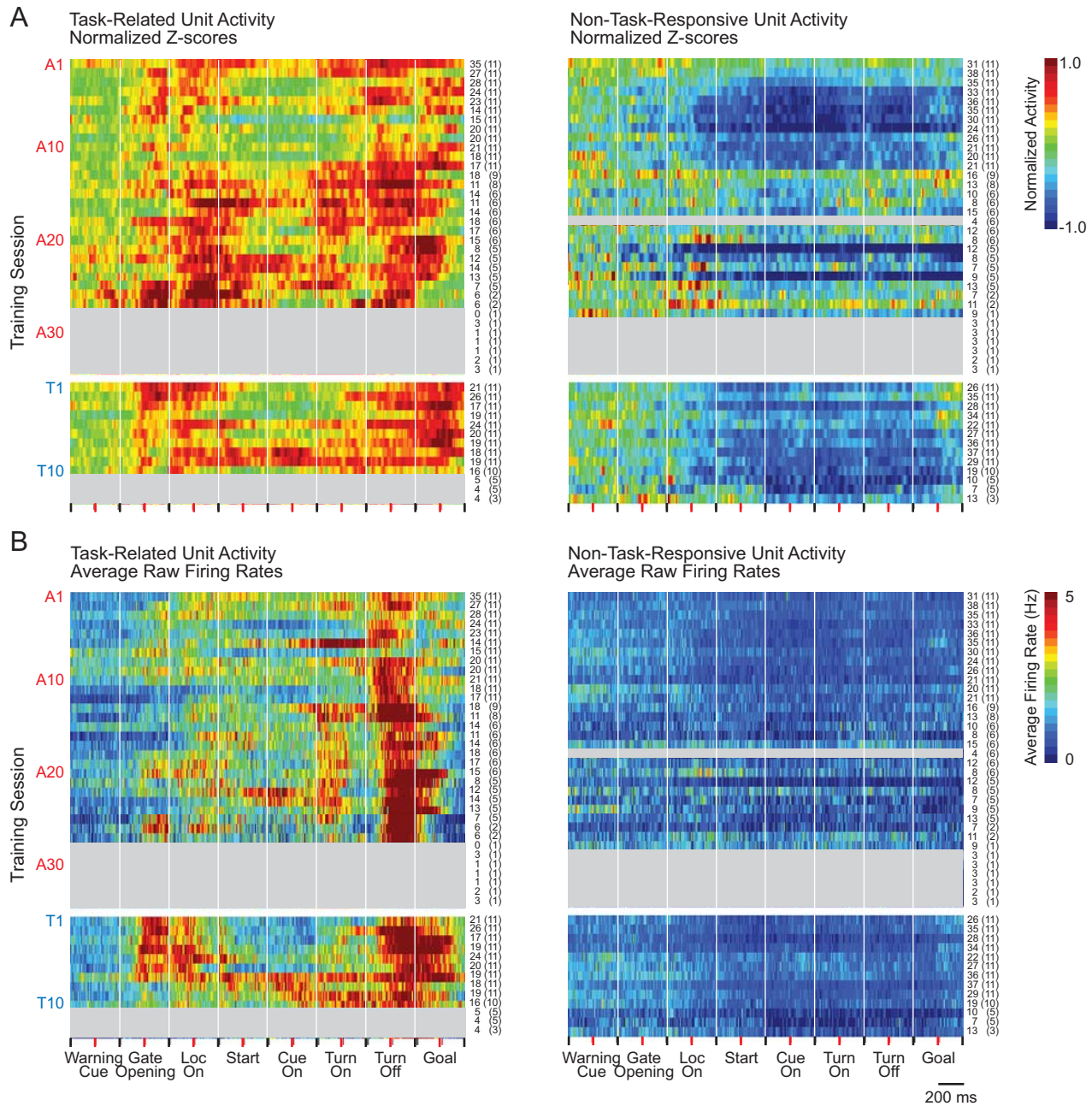


Supplemental Figure 3. Ensemble activity patterns calculated with different averaging procedures. *A* and *B*: Pseudo-color plots showing average raw firing rates (*A*) and average spike counts normalized to the minimum and maximum values for each individual unit (*B*) for MS units with (*left*) and without (*right*) task-related phasic responses. Numbers of units included in each stage are shown at right. *C* and *D*: Average raw firing rates of task-responsive (*C*) and non task-responsive (*D*) units during 200-ms before and after each event calculated for 6 training phases.

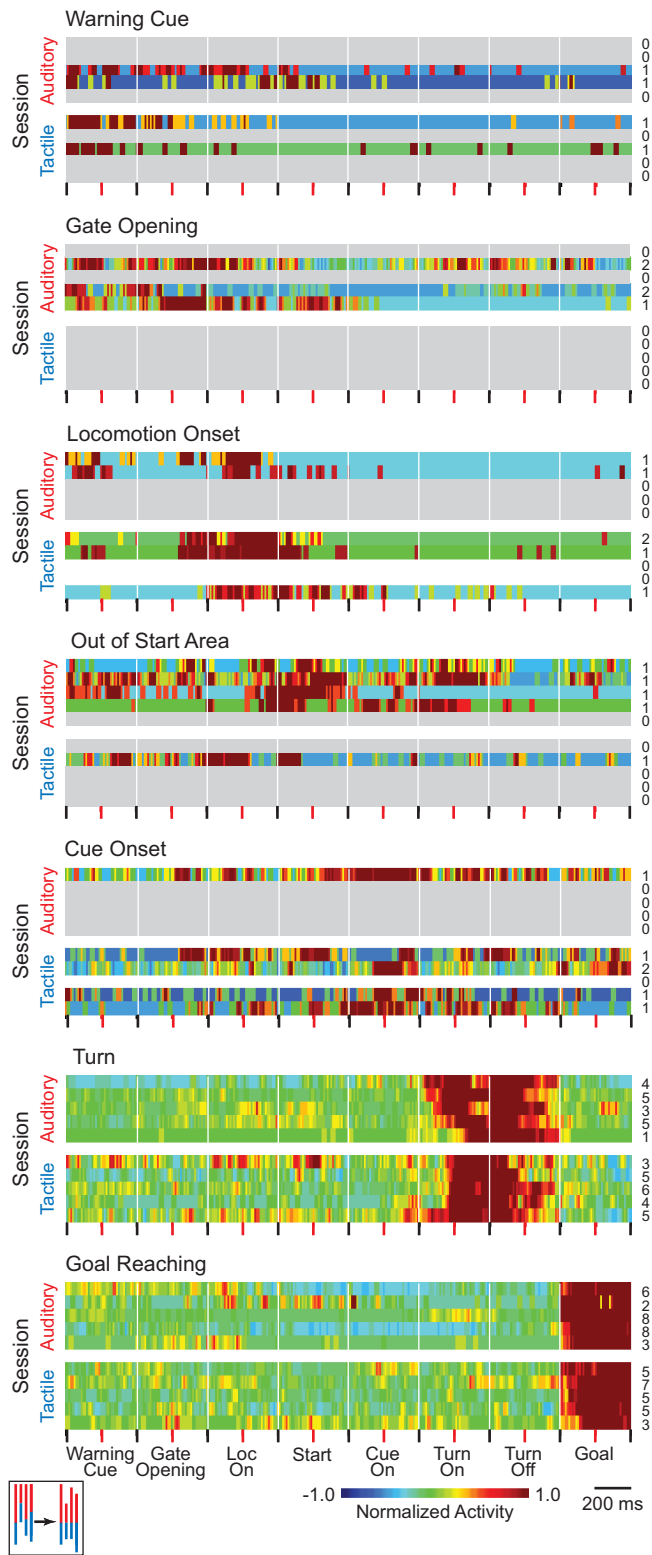


Supplemental Figure 4. Average ensemble activity of task-related MS units for trials with correct behavioral responses (*left*) and for trials with error responses (*right*). Plots show ensemble neuronal activity calculated with average Z-scores normalized to pre-trial baseline activity (A) and average raw firing rates (B).

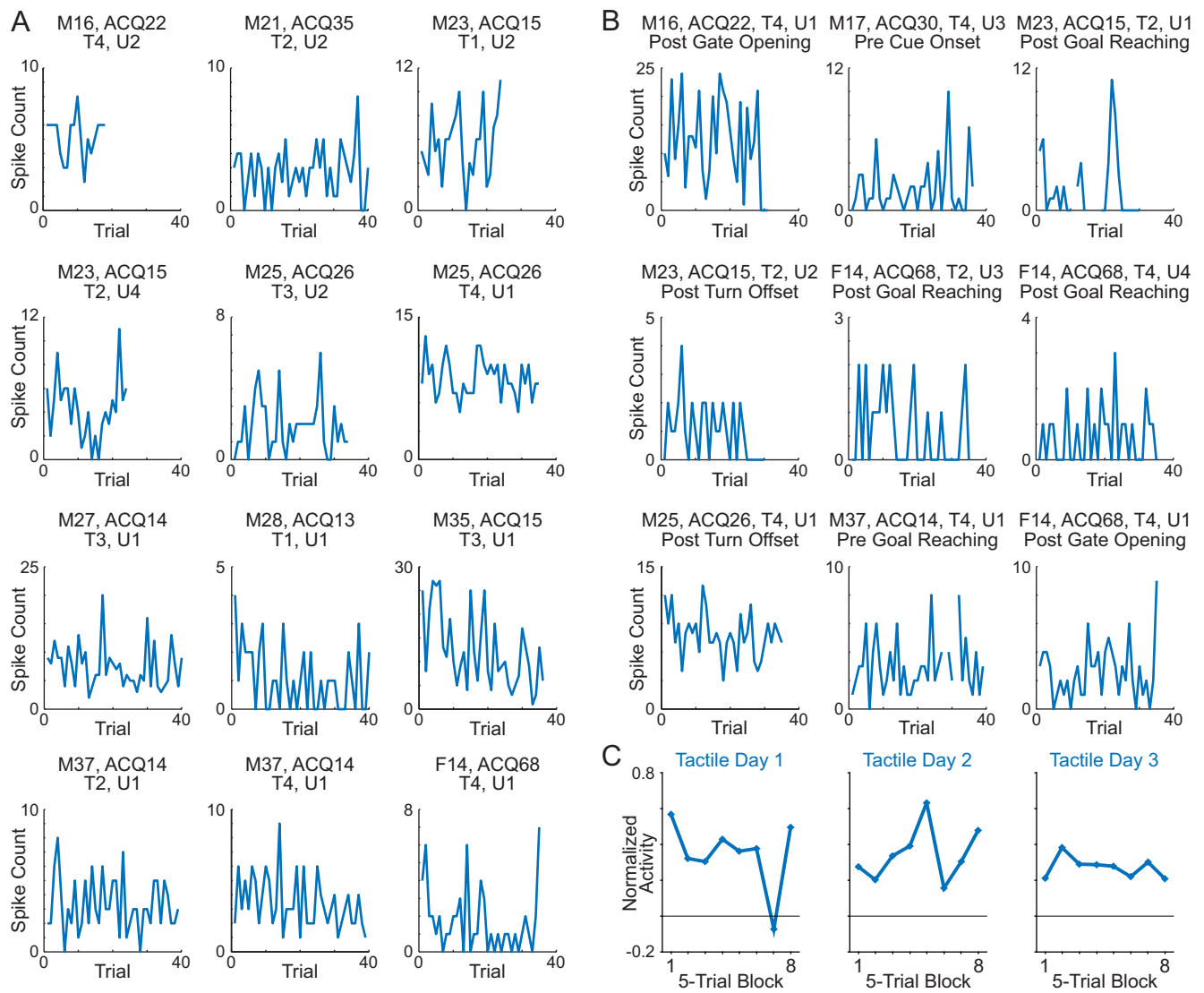




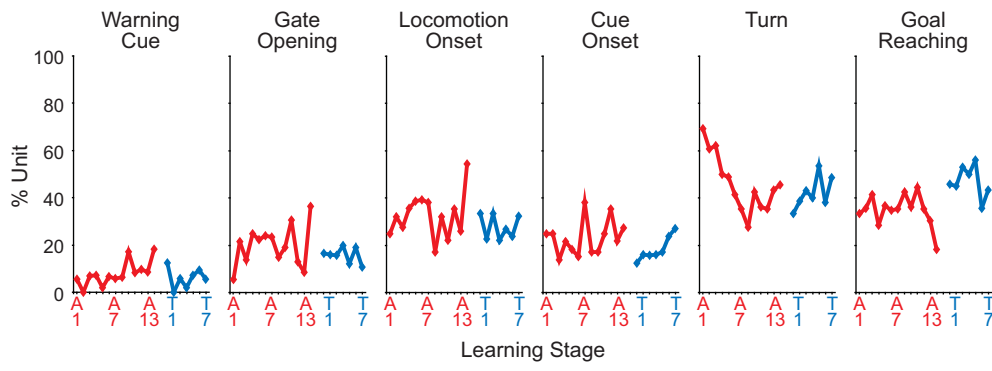
Supplemental Figure 5. Day by day average ensemble activity of MS units with (*left*) and without (*right*) task-related phasic responses during training on the auditory followed by tactile versions of the T-maze task, plotted with Z-scores normalized to the pre-trial baseline activity (A) and average raw firing rates (B). Gray areas indicate sessions with fewer than 6 units.



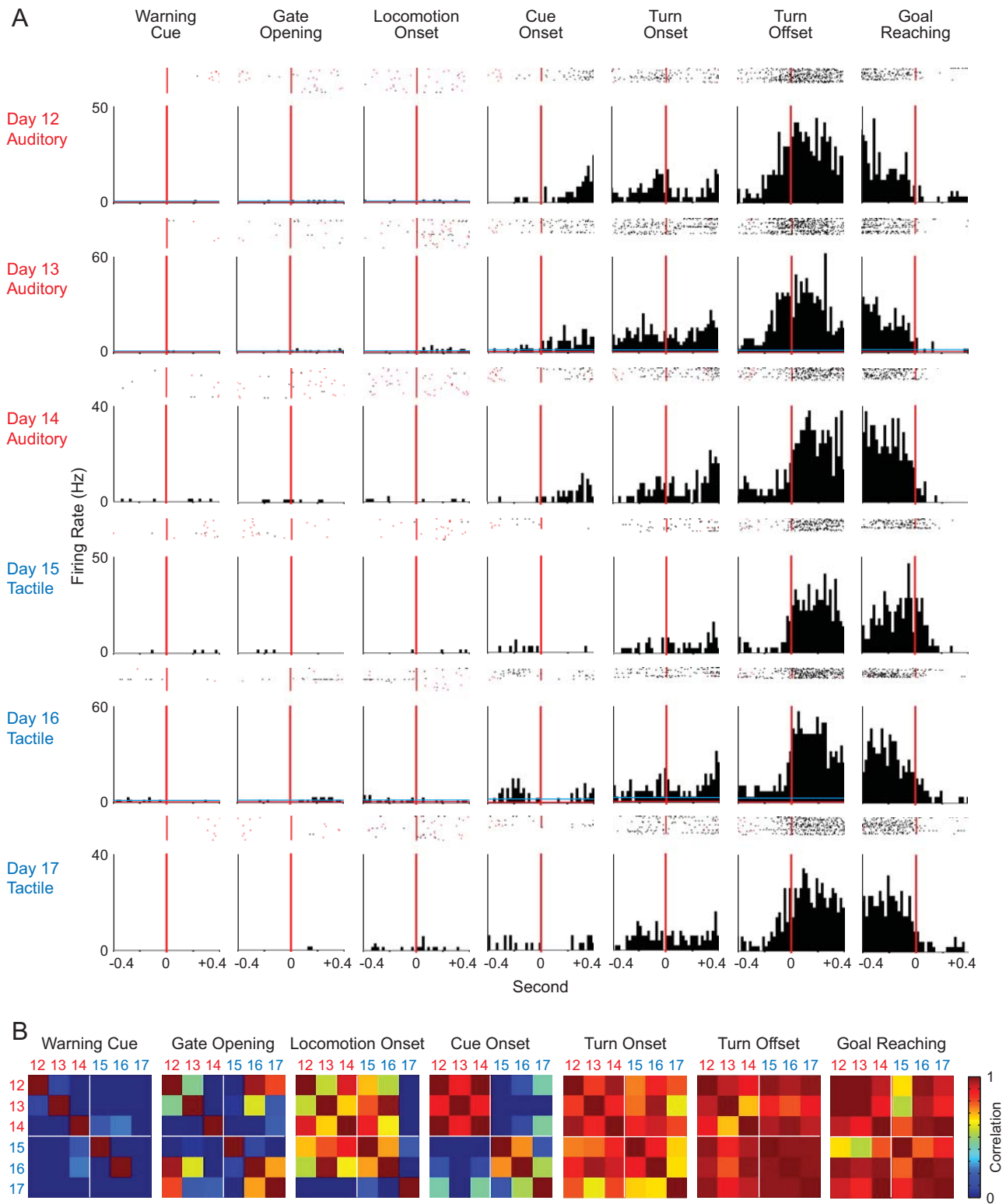
Supplemental Figure 6. Average normalized ensemble activity of MS units with responses exclusively to warning cue, opening of start gate, locomotion onset, out of start area, cue onset, turn and goal reaching during the last 5 auditory sessions and the first 5 tactile sessions. Gray area represents stages with no units with the particular task-related responses.



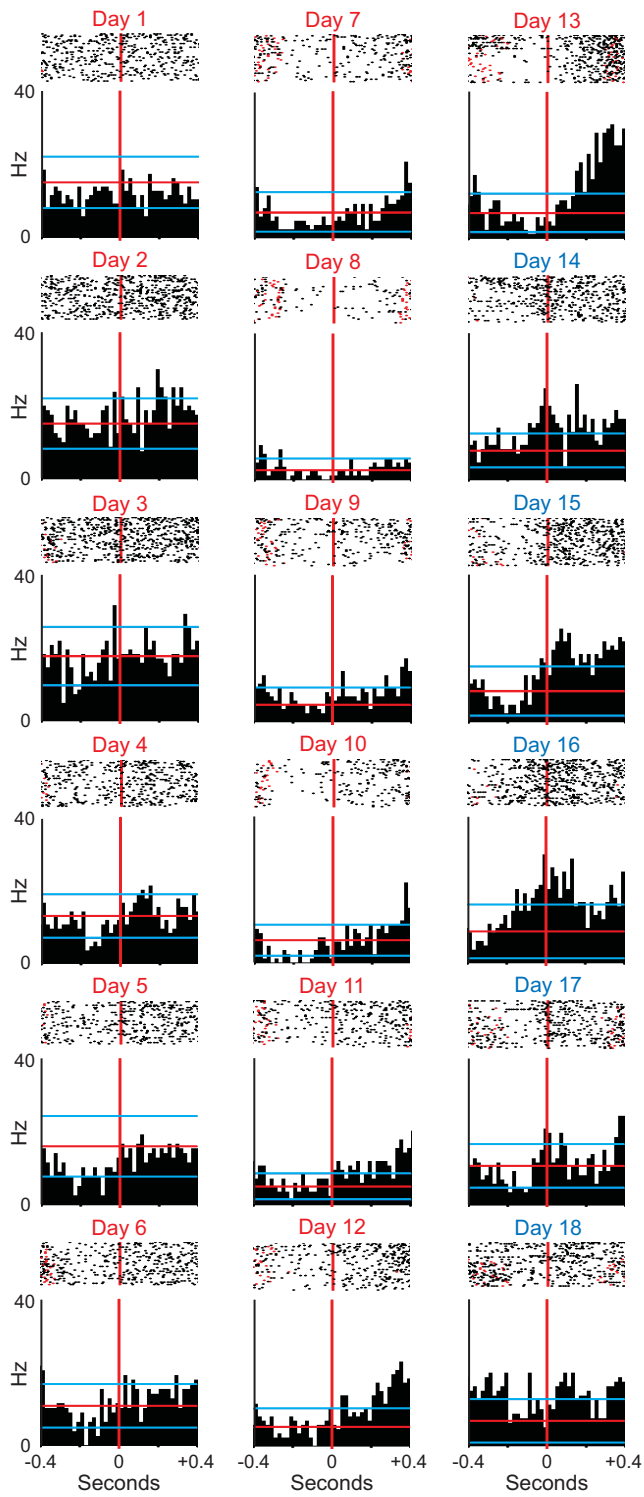
Supplemental Figure 7. Changes in spike counts during tactile sessions following the cue switch, in which large behavioral changes occurred early in the 40-trial session. (A) Responses of 12 individual FF units during the 200-ms post-cue period. (B) Samples of the MS (top 2 rows) and FF (bottom row) units for which significant change in activity was detected at the cue switch. (C) Average ensemble activity of task-related FF units during the 100-msec post-cue period in 5-trial blocks of the first 3 tactile sessions.



Supplemental Figure 8. Percentages of MS units that responded to each task event, as labeled, relative to all task-related MS units.



Supplemental Figure 9. A second example of a unit tracked across the task-version switch exhibiting loss of post-cue activity that occurred in response to the switch ( $P < 0.01$ ). Like the unit shown in Fig. 9, this unit also maintained relatively stable responses at turn end and goal reaching across the cue switch ( $P = 0.07-0.91$ ). *A*: Average activity during peri-event periods, as labeled, for the last 3 sessions of auditory training (days 12-14) and the first 3 sessions of tactile training (days 15-17). *B*: Correlation matrix of activity patterns during each peri-event period.



Supplemental Figure 10. Cue-related responses of a putative single unit of FF type tracked across the cue switch. Activity during the pre- and post-cue windows was greater during tactile sessions than auditory sessions ( $P < 0.05$ ).