1. Additional analysis in ‘switched’ and ‘modulated’ units

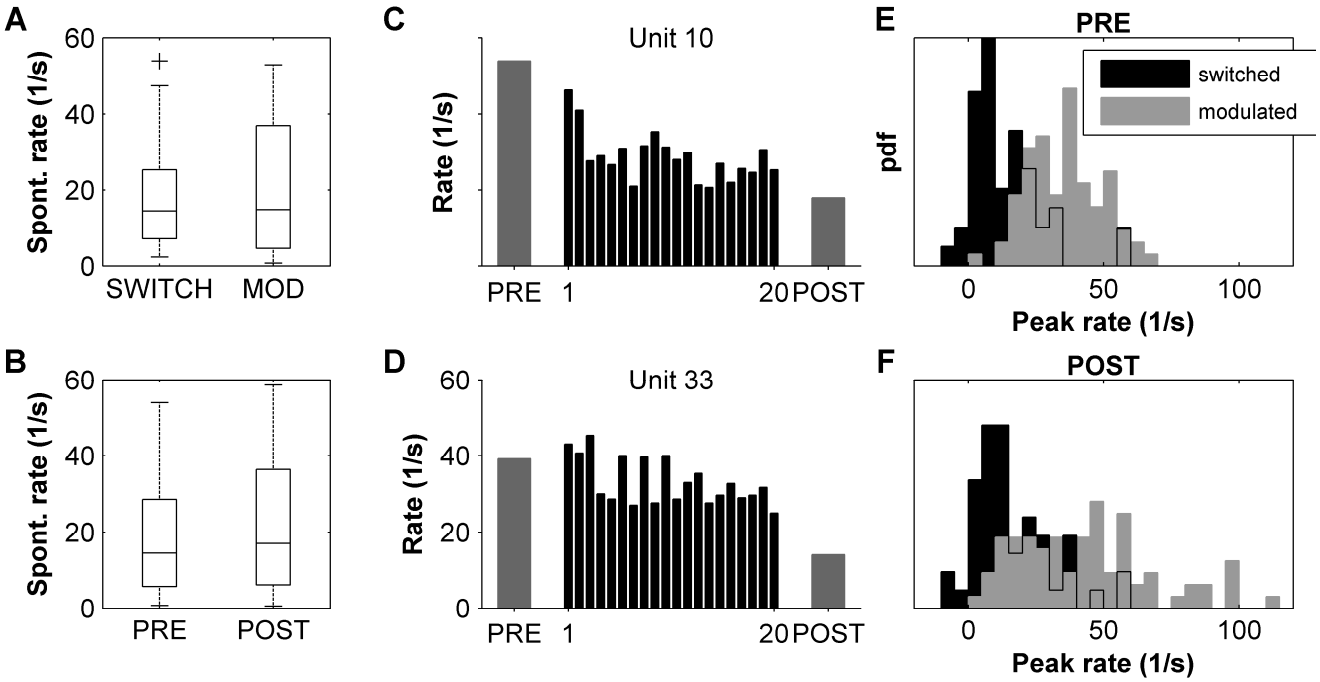
1.1. Spontaneous and response rate for 'switched' and 'modulated' units

We first compared the spontaneous firing rates of switched and modulated units as computed during three seconds preceding the stimulus onset in the PRE phase – i.e. before conditioning – and averaged across all 5 odors and across trials (supplemental Fig. 1 A). The median rates were not significantly different for both groups (P=0.91, Wilcoxon rank sum test) with mean rates of 17.7 spikes per second (switched units) and 20.2 spikes per second (modulated units). Additional analyses of spontaneous EN activity with respect to serial interval correlation and classification of ISI distributions is provided in Farkhooi et al. (2009).

Second, we compared overall spontaneous firing rates of ENs and pooled switched and modulated units (N=35) before and after conditioning (Fig. 1B). The distributions were not significantly different in the two phases (P = 0.58, Wilcoxon rank sum test).

Third, we tested for significant changes of the spontaneous firing rate in single units across all 20 subsequent acquisition trials (10 trials CS+ and 10 trials CS-) by testing for a significant correlation of spontaneous firing rates and trial order using Spearman's rank-order correlation coefficient. We found that 8 of 35 units showed a significant correlation ($P<0.05$, two-sided test). Five of these units decreased their spontaneous firing rate as shown for two examples in Fig. 1 C and D, while 3 units increased their spontaneous firing rate during conditioning. Increasing and decreasing spontaneous firing rates were found in switched units (N=6) as well as in modulated units (N=2).

Fourth, we compared the peak response rates during odor stimulation. For odors that evoked a significant response we subtracted the baseline firing rate during 3s preceding the stimulus from the peak firing rate within the response interval from 50-350ms after stimulus onset. Supplemental Figs. 1 E and F show the distributions of peak response rates in the pre and the post phase, respectively. The median peak rates of switched and modulated units were significantly different during both, the pre and the post phase ($P<10^{-3}$, Wilcoxon ranksum test).

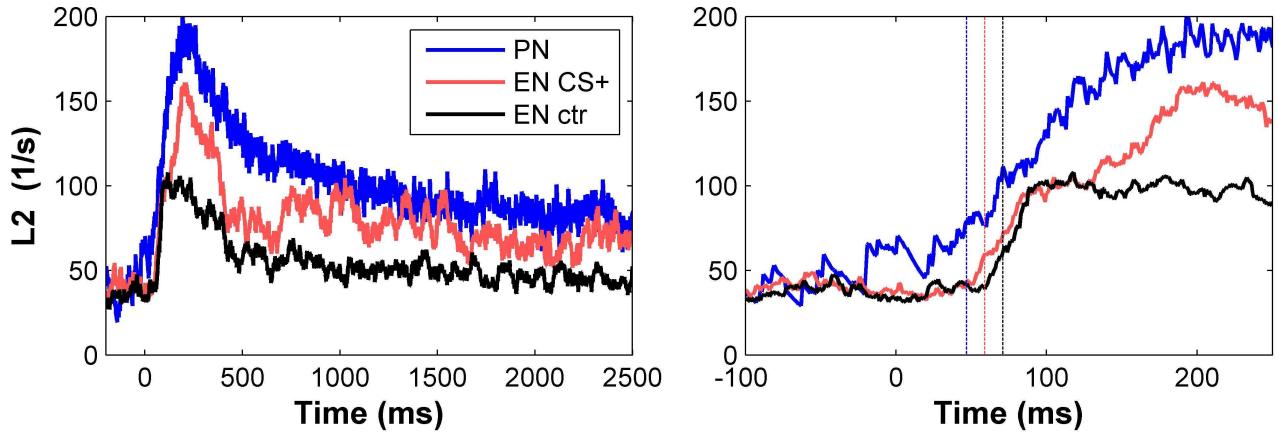


Supplemental Figure 1: Spontaneous rates and peak response rates for switched and modulated units. (**A**) Box plot of average spontaneous firing rates of switched and modulated units. Median rates were not significantly different (see text). (**B**) Spontaneous firing rates pooled for switched and modulated units were not significantly different in the pre and the post phase (see text). (**C**) Gray bars show spontaneous firing rates before stimulus onset averaged across all 50 trials (10 per odor) before (PRE) and after (POST) conditioning for unit 10 (same unit as in Fig. 3A in the main body of the article). Black bars show the firing rate for all 20 subsequent acquisition trials (10 trials CS+ and 10 trials CS-) in the order of appearance. Their ranks are significantly correlated with their order of appearance (see text). (**D**) Same as in (C) for the modulated unit 33. (**E**) Histogram of peak response rates for switched and modulated units in the pre phase and in the post phase (**F**). Median response rates were significantly different in the two groups and in both experimental phases (see text). Average peak response rates were 14.8 and 17.1 spikes per second pre-conditioning and 36.1 and 43.8 spikes per second post-conditioning for switched and modulated units, respectively.

1.2. Dynamics of population encoding in projection neurons (PNs) and mushroom body extrinsic neurons (ENs)

In Fig. 8 of the main body of this manuscript we show the dynamics of odor representation in the EN population firing rates. Odor representations became significantly different after ~60-70ms (59ms for CS+ vs. control odors and 71ms among control odors). To make the comparison between the population response dynamics of neurons providing the input into the mushroom body (MB) (PNs) with the population response dynamics of neurons reflecting the output of the MB (ENs) we added a dataset of 17 uniglomerular projection neurons (PNs) published in Krofczik, Menzel, and Nawrot (2009). Our results shown in supplemental Fig. 2 suggest that odor representations follow a similar dynamics at the level of PNs and ENs. Note that the PN population (N=17) is smaller than the EN population (N=38), i.e. the Euclidean distance per neuron is considerably larger in PNs than in ENs. Euclidean distance for PNs becomes significantly different from baseline at around 45-50 ms, i.e. some 10-20ms before the EN population responded to the CS+ and the control odors. Note, that in both experimental setups the odor travel time from the computer controlled

valve to the bee's antennae had to be estimated from the geometry of the setup and air speed and thus is subject to a possible estimation error.



Supplemental Figure 2: Dynamic odor representation in projection neurons of the AL and MB extrinsic neurons. *Left*: Euclidean distances between odor-encoding population rate vectors. The black curve (control odors) and red curve (CS+ vs. control odors) as in Fig. 8C. The blue curve illustrates a population response of N=17 projection neurons averaged across 3 different odor pairs (blue; Krofczik, Menzel, and Nawrot 2009). *Right:* Same as left but zoom into the first 250 ms of population responses. Vertical lines indicate significance of odor representation (baseline + 5 SD) following the same coloring. The PN population response started around 10-20 ms earlier than the EN population responses to CS+ and control odors.

2. Stability and quality of single unit recordings

2.1. Spike sorting and unit separation

The waveforms shown in supplemental figures 3-6 were recorded in four different bees (Bee 67, 73, 83 and Bee 87) and illustrate the data quality and the separation of spike shapes that were sorted as belonging to different single units. The first unit of each of these bees is presented in Figure 3 in the main body of this publication to introduce switched (recruited and dropped) and modulated (increased and decreased) units. To separate single unit activity we always used the differentially recorded channels (cf. Fig. 1 of the main body). From the channel showing the best signal to noise ratio in each bee, mostly two different units were detected. To convince ourselves that the recording conditions had not changed during the total of ~5 hours recording, we compared the waveforms between the pre conditioning phase and the post conditioning phase (upper panels in supplemental figure 3-6). In addition, we checked the unit separation using principal component analysis (PCA). Both features are provided with the Spike2 software (Cambridge Electronic Design, Cambridge, UK) and were used during application of the semiautomatic template matching method for spike sorting. However, if the visualization of the different units in the post conditioning phase did not allow a separation equal to that during the pre conditioning phase, we used the related units to analyze initial response characteristics only. Therefore, we had to remove 6 units from the pre post comparison analysis. As an additional characteristic, we

visualized the inter spike interval (ISI) distributions for each unit (supplemental Fig. 3-6). Therefore we used 3 minutes of spontaneous activity which we recorded before odor stimulation.

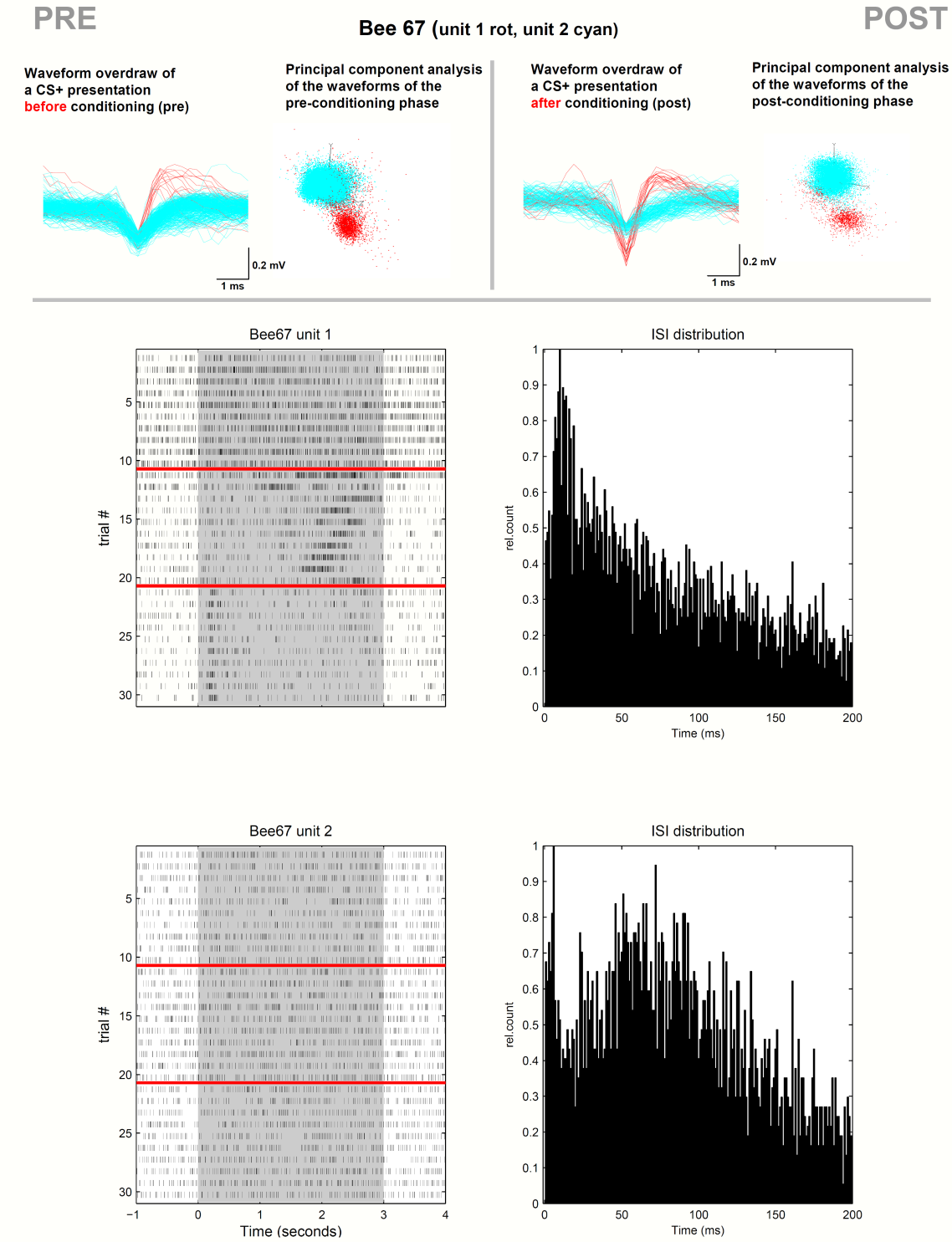
In order to prevent electrode drift which is a major obstacle for stable long term recordings we glued the recording electrode to the brain surface using non toxic two component silicon (for details see Materials and Methods). In addition the space between the brain and the cuticle of the preparation window was completely filled with the silicon. This novel procedure successfully stabilized our recordings and prevented the bees for drying-out. It allowed stable separation of single units during the complete experiment in most of our recordings. In some exemplary experiments we could establish stable recordings for more than 24h (not shown).

2.2. Comparison between extracellular recordings of KCs and ENs

Supplemental figure 7 shows the different extracellular recorded signals from Kenyon cells (KCs) and ENs. To visualize the recording position we usually dipped the tip of the electrode into a fluorescence dye (Lucifer yellow). To record KC activity we inserted the electrode dorso-ventrally between the lips of the median calyx. We drove the electrode for nearly 100 µm along the peduncle. The different stacks (anterior-posterior) in supplement figure 7 shows the insertion channel in three subsequent stacks. The right KC stack (180-230 µm) shows that the dye marked backwards a part of the posterior median Calyx were cell bodys of KCs are located. ENs we recorded as described in the Materials and Methods section of the main body. The visualization of both recording positions allows a reference to the related neuron type.

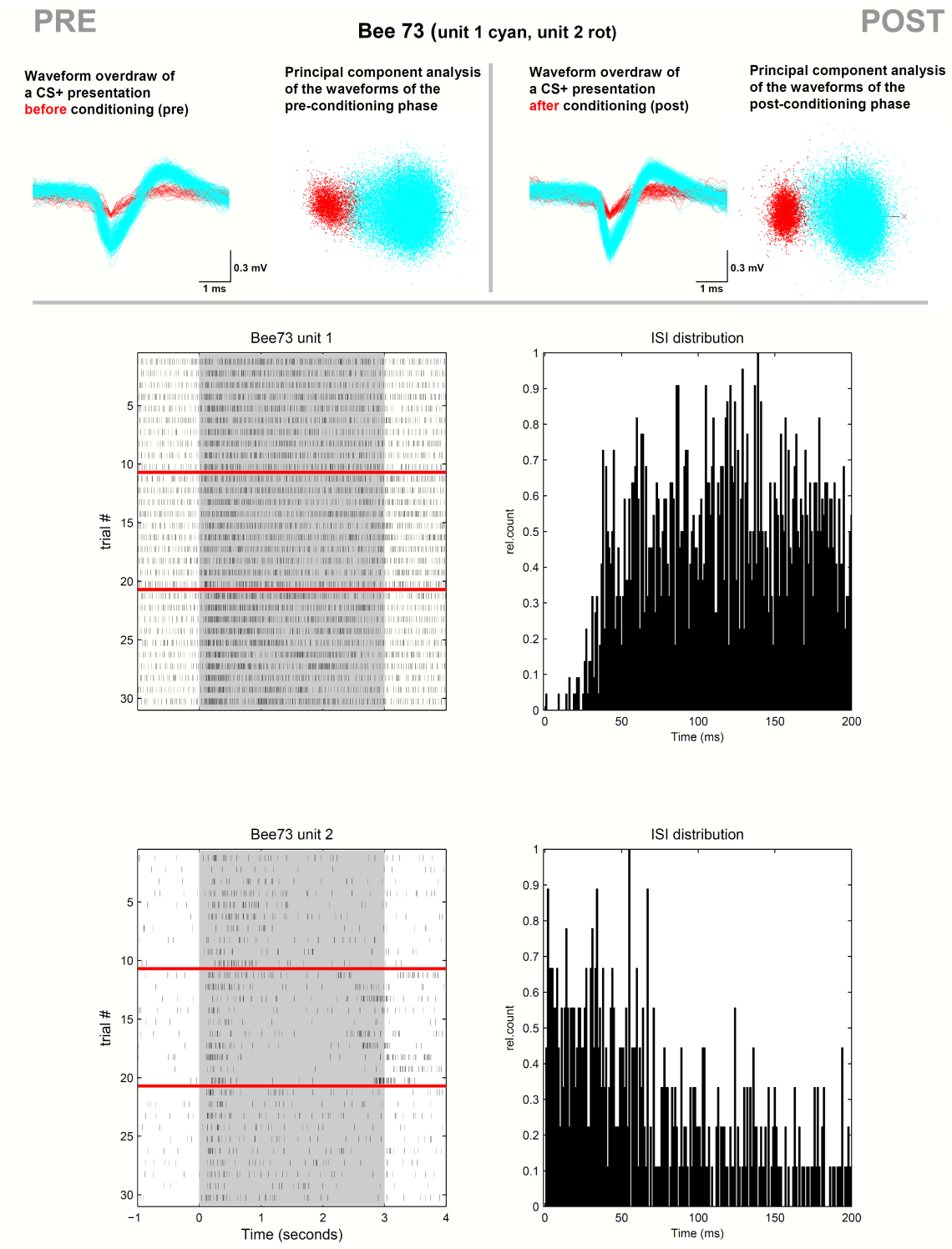
The neurite diameter of ENs are comparably big in the range of 5-10µm and very close to the size of the single electrode wires used in our experiments (14µm). This relationship in size might be the reason for the high signal to noise ratio and the comparably small number of units that were separated from a single EN recording channel (max. 3 units could be separated).

In contrast, the neurite diameter of KCs is rather small (<0.5 µm). Using the same type of electrode to record from a KC population resulted in a rather noisy signal dominated by small spikes presumably from a large number of different KCs. The extracellular recorded waveforms of KCs and ENs have two distinct characteristics. First, the duration of EN spikes (~3ms) is about twice as long as the duration of a KC spike (~1.5ms). Second, KC spikes generally assume lower peak to-peak values (supplemental figure 7 B and C).

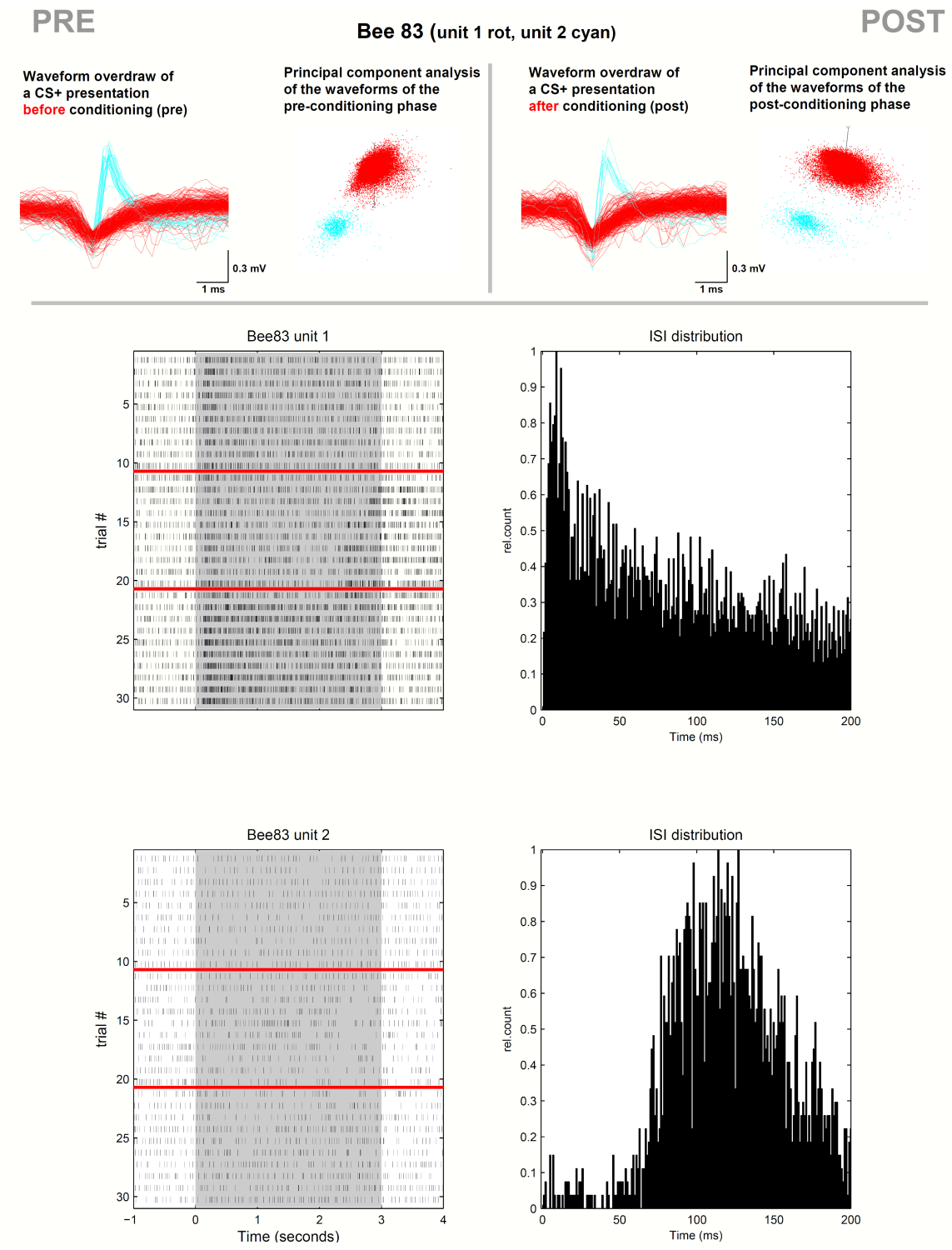


Supplemental Figure 3: Example of the unit separation of Bee 67. After spike sorting using the template matching technique in Spike2 (cf. Methods) two different waveforms were separated at the same differential electrode channel. The single events of each kind were interpreted to be produced by one neuron and therefore called unit. The separation of unit 1 (red) and unit two (cyan) of bee 67 is visualized as 2D projection of the three most dominant principal components. The left side (PRE) illustrates the situation before odor conditioning and the right side (POST) after odor conditioning. Note that there were at least 3 hours in between. For both

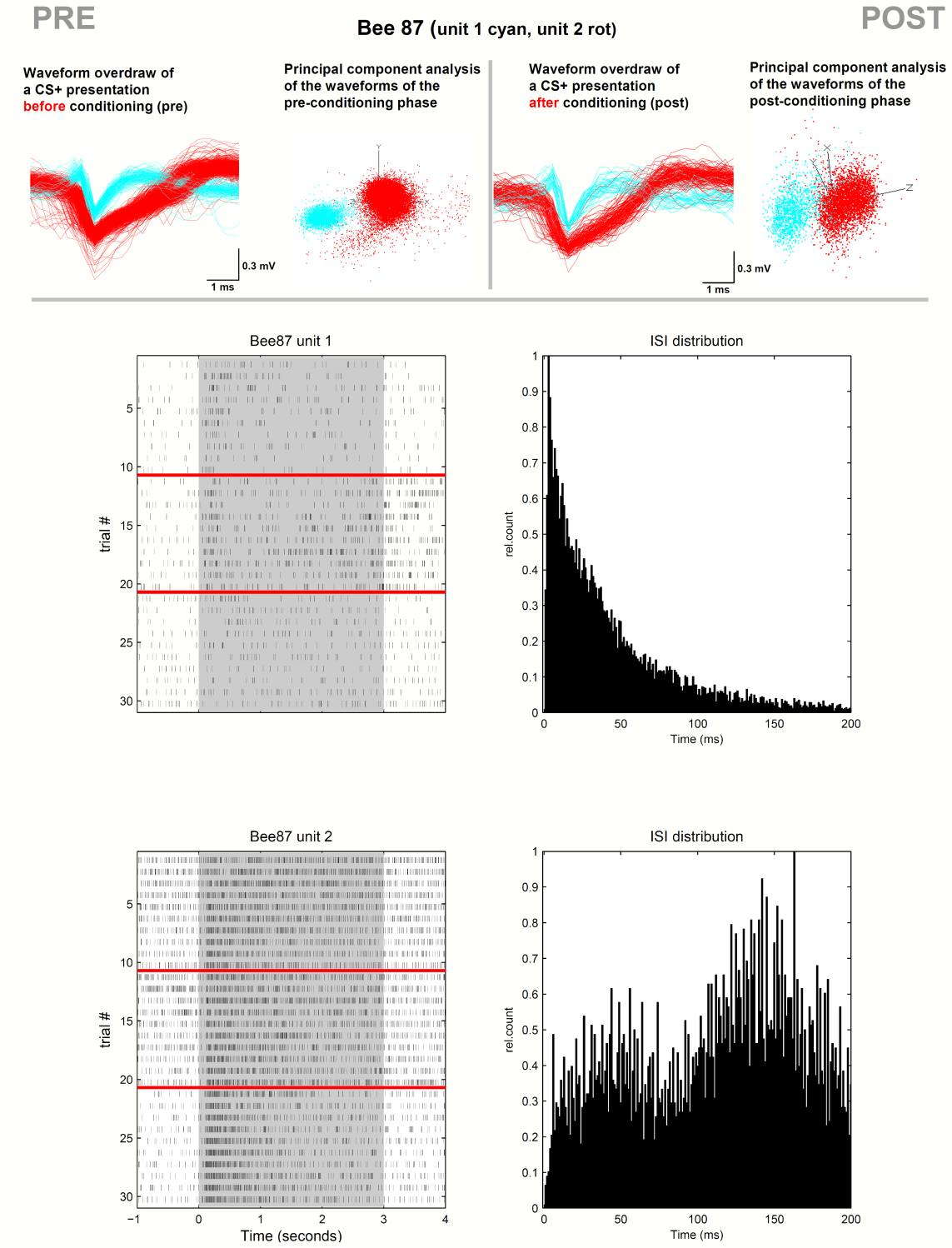
experimental phases we show all single spike waveforms during 10 seconds of recording including the three seconds of odor stimulation with CS+. The representation by principal components includes all spikes from the complete PRE and POST conditioning phases, respectively.
The spike raster displays 'Bee67 unit 1' and 'Bee67 unit 2' illustrate the spike time dynamics of each unit during the complete experiment including all phases. Each plot includes the 30 CS+ stimulations (10 trials pre-, 10 trials during and 10 trials post- conditioning). The experimental phases were separated by red lines. The time of the three seconds lasting odor presentation is marked by the gray background. Beside each dot display the inter spike interval distribution (ISI) of the spontaneous firing activity of the related unit is shown.



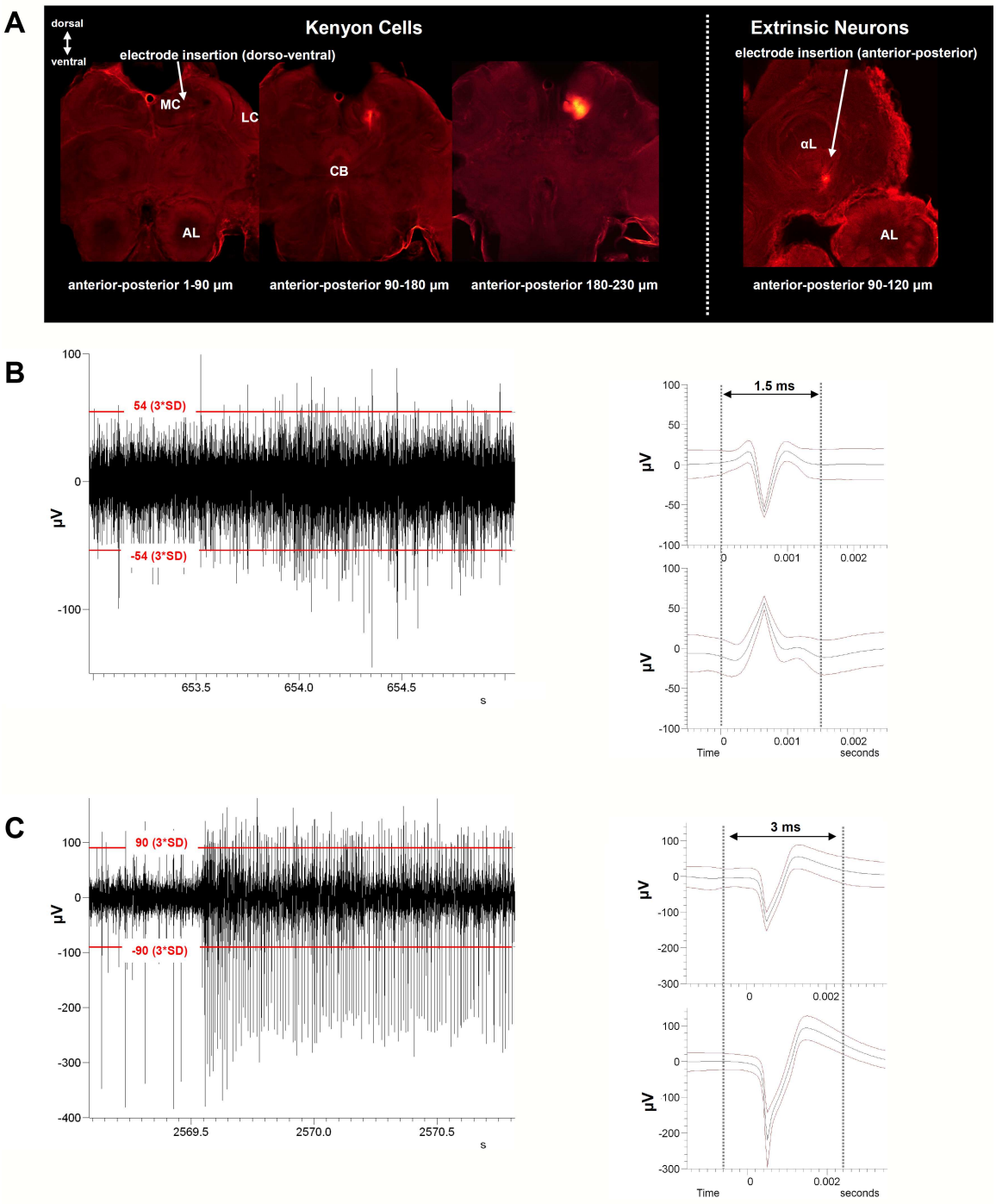
Supplemental Figure 4: Example of the unit separation of Bee 73. Procedures and conventions as in Figure 3.



Supplemental Figure 5: Example of the unit separation of Bee 83. Procedures and conventions as in Figure 3.



Supplemental Figure 6: Example of the unit separation of Bee 87. Procedures and conventions as in Figure 3.



Supplemental Figure 7: Comparison of extracellular recordings from ENs and KCs. (A) Visualization of the recording position of Kenyon Cells (left) and Extrinsic Neurons (right). To record KCs extracellular we inserted the electrode dorso-ventral along the pedunculus starting at the center of the Median Calyx (MC). The series shows the penetration channel in different depth. At a depth between 180-230 µm the fluorescent dye (Lucifer yellow) marked retrograde the posterior Area of the median Calyx. For comparison at the right the electrode insertion point to record ENs (cf. methods). (**B**) Differential extracellular recording channel of KC appear to be noisier and the detectable spike waveforms (right) show a shorter duration and a smaller amplitude as compared to the EN spikes shown in (**C**). The spike waveforms are displayed as mean ± standard deviation. The KC-unit width is nearly half the size (1.5 ms) compared to the EN-unit width (3 ms).

3. Monitoring the behavior

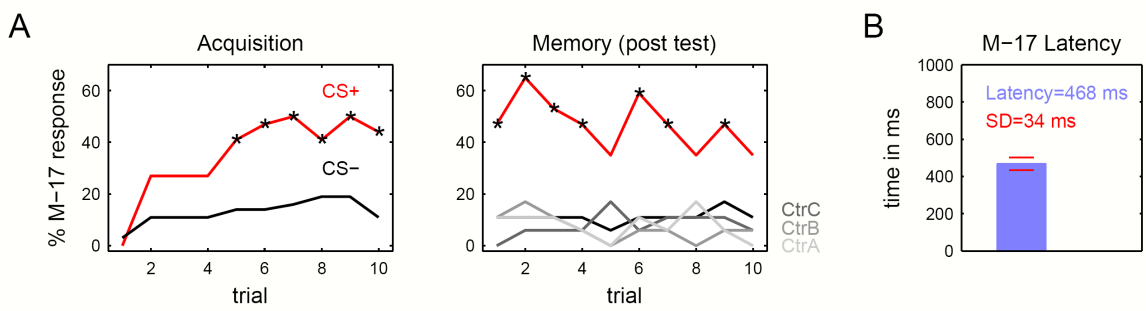
The extension of the bees proboscis (PER) was used as a behavioural measure of associative responses. PER was monitored by the myogram of muscle M17, a muscle that is involved in proboscis extension (Rehder, 1987). During the acquisition phase a conditioned response was detected if the activity of the muscle M17 started right after the CS+ onset before the US was presented. During the later test phase, we monitored the activity increase after odor presentation. Differences between CS+ and CS- trials were tested using a G-test for contingency ($p < 0.05$).

3.1. Normal learning performance during extracellular recording

To investigate changes in neural response due to learning we devised a second series of conditioning experiments. Five odors were tested before and after differential odor conditioning. During the conditioning phase, two of the five odors were presented, one was rewarded, the other not rewarded. The bees showed normal learning behaviour and expressed a typical learning performance during the recordings (supplemental Fig. 8) indicating that they discriminated the CS+ and the CS- significantly after the 4^{th} conditioning trial (supplemental Fig. 8A left; G-test, $p<0.05$; df=1). Three hours after acquisition retention scores were significantly higher for CS+ than for CS- during the first trial, a differentiation that remained relatively stable during the following 9 retention tests (Fig. 8 A right; $G_{trial1}=5.37$; $p=0.02$; df=1). Control A, B and C indicate three control odors that were not used during acquisition (cf. methods). Note; the phenomenon of extinction occurs slowly and rather as a kind of trend. The 9^{th} CS+ repetition of the post test is still significantly different from the CS- and the control odors.

3.2. Behavioral response latency

Next we asked how long bees need until they extend their proboscis after odor stimulus onset. Therefore we calculated the mean latency between antennal odor stimulation and the first M17 spike. We included 8 bees that responded in at least 5 of the 10 CS+ trials (65 trials in total). The delay between opening of the magnetic valve and the arrival of the odor plume at the antennae was determined as 37 ms and subtracted from the latency (cf. methods). On average, the muscle activation occurred at 468 ± 34 ms. This is the time that the bees needed to decode the odor stimulus, to form a decision, and to finally extend their proboscis (supplemental Fig. 8B).



Supplemental Figure 8: Differential conditioning and response latency (**A**) The proboscis extension was monitored by recording muscle potentials from the muscle (M17). During acquisition bees learned to discriminate between CS+ and CS- (n=36). Discrimination became statistically significant from the 5th trial onward (significance indicated by a star; G-test; $p<0.05$; df=1). Three hours after acquisition animals were exposed to 10 retention tests for each of 5 odors (n=17). In most trials CS+ responses were significantly higher than those to the CS- and any control odor (CtrA to C) as indicated G-test; $p<0.05$; df=1). The differences in numbers of tested animals (n=36 and n=17) is based on an additional subset of bees that were recorded during acquisition only (cf. methods). (**B**) Mean latency between odor reception at the antennae and the M17 response onset (blue bar) as calculated from the CS+ responses of 8 bees (65 trials) (cf. Fig 3, inset iii and methods). Red lines mark standard deviation.