Neural correlates of side specific odour memory in mushroom body output neurons

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Supplementary Material

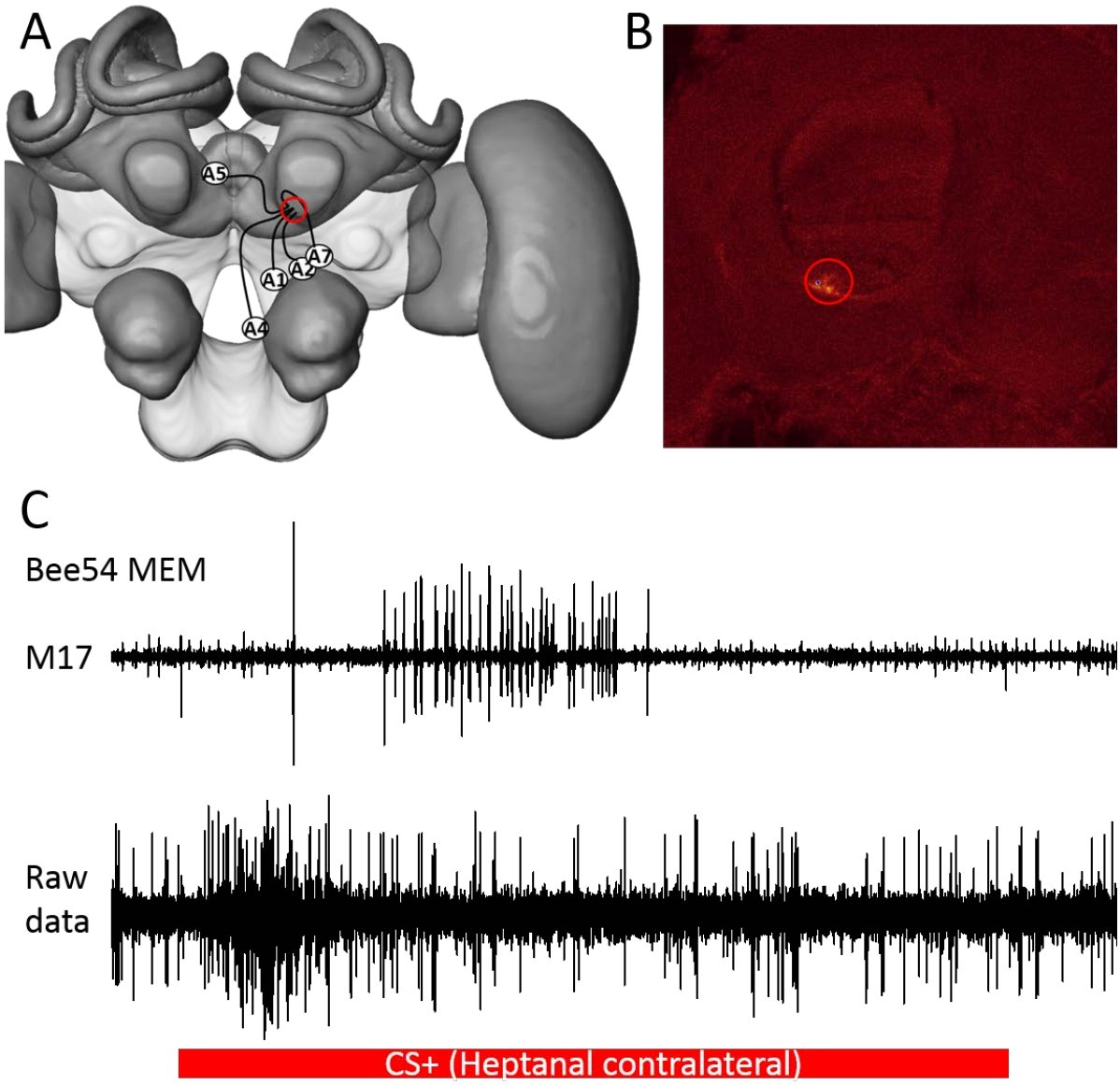
S1: Recordings from mushroombody output neurons

To record from mushroom body output neurons (MBON) in one brain side of behaving honeybees we used a well-established extracellular recording technique. Using this approach stable long-term measurement of neuronal spiking activity for many hours has been documented for antennal lobe projection neurons [1-3] as well as MBONs [3-7] in the honeybee. This is a prerequisite for the comparison of neuronal activity before, during and after applying the unilateral training protocol to the antenna contralateral to the recording electrode position. We inserted the electrode at the ventral aspect of the left alpha-(vertical) lobe at a depth between 150-250 µm (supplementary figure S1A, B). Following the nomenclature introduced by Ryback and Menzel [8] the recorded neurons can be related to the A1, A2, A4, A5 and A7 cluster of the mushroom body extrinsic neurons (supplementary figure S1A). To control for a correct placement we marked the recording electrode insertion (for details cp. [1, 6] and related supplements) with, e.g., Lucifer yellow (supplementary figure S1B). MBON neurites in that area are comparably thick (~10µm) and induce pronounced spike shapes in our recording electrodes (supplementary figure S1C), which can be reliably sorted to obtain single unit activity ([cp. supplements in [1, 6]). In contrast, other neurons in that area, in particular Kenyon cells, have neurite diameters of <0.5 µm and induce therefore less pronounced action potential waveforms as shown in our earlier publication [6]).

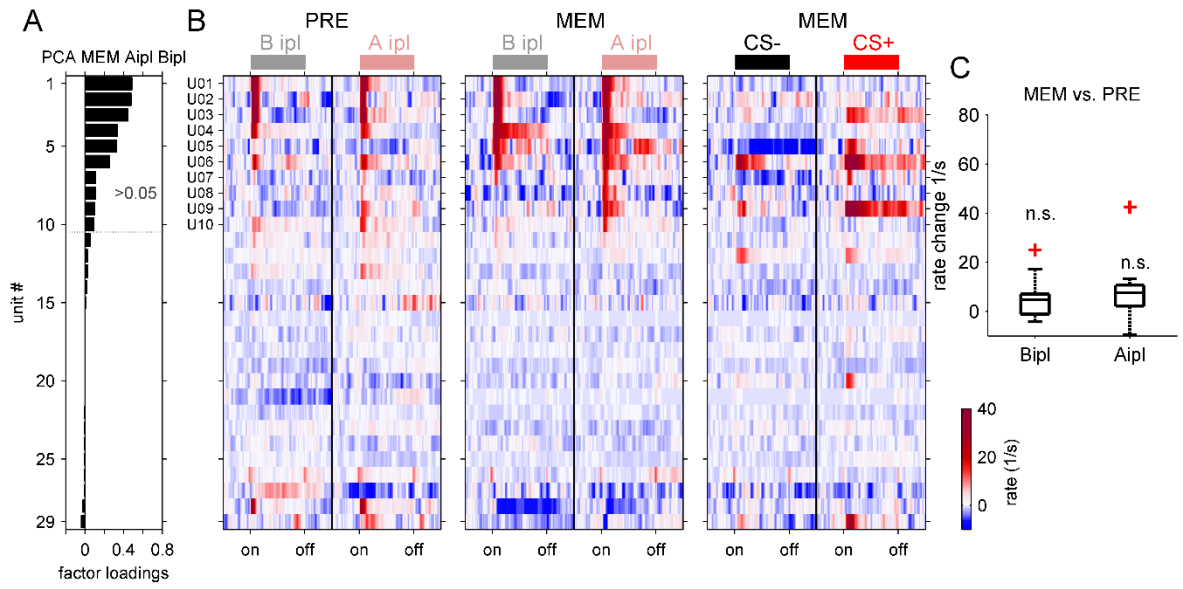
To observe simultaneously neuronal activity and the conditioned response behaviour of the bees we recorded the M17 muscle activity (cp. method section). Supplementary figure S1C shows an example of a M17 response trial, which was established during the memory phase (MEM) when the animal had associated an odour side compound stimulus with the sucrose reward. Note, this is the very same bee from which unit U01 was extracted (shown in Figure 4).

S2: Ipsilateral induced odour reponse activity in MBONs was not significantly affected by contralateral odour reward association

The vizualization after principal component analysis (PCA) in Figure 3 illustrates that the ipsilateral odour representation was not affected. However, the calculation of the Euclidean distances in figure 2C shows a small increase of the ipsilateral odour separation during the MEM phase. To analyse if this activity increase reflects an recruitment of single units as it was the case due to contralateral stimulation we applyed a PCA separrratly based on the population vectors representing ipsilateral induced activity during the MEM phase (supplemantry figure S2).



Supplementary Figure S1. Recording extracellular mushroom body output neurons. A) 3D image of the honeybee brain (http://www.neurobiologie.fu-berlin.de/beebrain). Neurons at the recording site can be related to the A1, A2, A4, A5 and A7 cluster of mushroom body extrinsic neurons (after Ryback and Menzel 1993). Red circle marks the electrode incertion position. **B)** Vizualization of the electrode position after recording using luciffer yellow. **C)** Memory test trial of Bee54 (cp. Fig 3F). The red bar indicates 3 seconds of contralateral stimulation with the reward associated stimulus. Raw data chanel which will be used furthrt to extract single unit activity. The M17 chanel indicates the actifity of the muscle inervating the proboscis.



Supplementary Figure S2. Ipsilateral MBON activity is not affeected by contralateral conditioning. A) Principal component analysis was performed limited to the population vectors reflecting ipsilateral induced activity during the MEM phase (middle heat map in B) and the single units were ordered with respect to their contributions to the variation of PC1 (factor loading). **B)** Trial-averaged firing rate profiles of all units (ordered as in A) of the PRE and the MEM phase for ipsilateral stimulation (A ipl, B ipl) and the MEM phase activity of the same units due to contralateral stimulation (CS-, CS+). **E)** The rate changes between the PRE and the MEM phase due to ipsilateral stimulation were calculated for the first 10 units (factor loading >0.05). The distribution of rate changes is shown in a boxplot; central mark indicates the median, edges indicate the 25th and 75th percentiles, whiskers extend to the most extreme data points, outliers were marked with red crosses. There was no significant response rate difference between the PRE and the MEM phase (signed rank test, $p<0.05$).

We ordered the units with respect to their factor loadings to PC1 (supplementary figure S2A). Overall, units which responded to ipsilateral stimulation before contralateral conditioning were also responding to ipsilateral stimulation during the MEM phase with few exceptions (e.g. U05). To analyse if there was a significant rate increase between the PRE and the MEM phase we extracted the units showing factor loadings >0.05 (as in figure 3) and computed the difference in firing rate for each single unit (supplementary figure S2C). Neither the A ipl (same odour as in CS+ compound) nor the B ipl (same odour as in the CS- compound) representation was significantly changed (signed rank test, $p<0.05$).

S3: Spike sorting

We recorded 3 minutes of spontaneous activity before starting with the stimulation protocol. The pair wise differentially recorded channels of the silicon anchored electrode were high-pass filtered (>600Hz) before we applied a semi-automatic spike sorting (template-matching) provided with the Spike2 software (Cambridge Electronic Design, Cambridge, UK). We calculated the mean signal and standard deviation (SD) during the spontaneous activity and set the thresholds for detecting spike events always above ± 3 x SD. Threshold-crossing events were used to compute templates of spike waveforms, which were subsequently used to assign individual spikes. To control for single unit separation we applied principal component analysis (PCA) of the detected waveforms and plotted

the inter-spike-interval (ISI) distribution using Spike2 (Cambridge Electronic Design, Cambridge, UK). Units which were separated during all experimental phases and showed ISIs > 1ms were used for further analysis (for details see supplemental Material in [6]).

Supplementary References

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