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Abrogation of dendro-dendritic release from olfactory bulb granule cells and its impact on odor discrimination

Promotionsfach: Anatomie und Zellbiologie
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With the goal of delineating out the contribution of granule cells, in the olfactory bulb, the synaptic output of these inhibitory inter-neurons, was rendered silent, and its impact on odor discrimination behavior was evaluated in mice. Synaptic release from granule cells was silenced using two independent strategies that introduced spatio-temporally defined genetic perturbations in the granule cells using recombinant adeno-associated virus (rAAV-1/2).

In the first approach, cre-recombinase expression in floxed Munc18-1 mouse line, enabled ablation of the vital synaptic protein Munc18-1 in the transduced granule cells of the olfactory bulb. In an alternative approach, botulinum neurotoxin light chain A (BoNT-A/LC) was genetically expressed in the olfactory bulb granule cells, to cleave the essential synaptic protein SNAP25, thereby leaving the affected granule cells synaptically silent. In each case, the genetic constructs of interest were designed to be co-expressed with a fluorescent marker. The gene constructs were first functionally verified in vitro and in vivo and then stereotaxically injected into the granule cell core of the olfactory bulb of adult male mice.

This was followed by assessing the odor discrimination behavioral phenotype (on a go-no paradigm, using automated olfactometers) which revealed a significant increase odor discrimination time needed by the silenced granule cell batch of mice compared with their normal litter mate controls using both approaches independently. This however was accompanied with no noticeable gross changes in neuronal morphology.

Olfactory discrimination time is measured in this study as the reaction time required by mice to distinguish one odor stimulus from another. The sensitive assay offers a millisecond temporal resolution of the mouse response time to reinforcing and non-reinforcing odor stimuli. The odor discrimination time required for simple odors was 242.5±8 ms for control mice and 306.5±14.2 ms for mice with BoNT-A/LC in GCs and for difficult binary mixtures was found to be 313.3±11.2 ms for control mice and 387.5±20 ms for mice with BoNT-A/LC in GCs.

Pooled results from two separate rounds of Munc18-1 ablation experiments showed that discrimination time required for simple odor pair was 228.6±9.12 ms for control mice and 344.5±14.7 ms for mice with Munc18-1 deleted in GCs and in case of binary mixture discrimination was found to be 314.0±11.2 ms for control mice and 398.6±13.8 for mice with Munc18-1 deleted in GCs. The pooled data, also notices a significant increase in time required for binary odor mixture discrimination with in mice with silenced granule cells compared to controls. As is the reported trend from previous studies, discrimination of binary mixture odor pair takes longer than for discrimination of simple odor pairs, after abolishing synaptic vesicle release. The spatio-temporally controlled abrogation of granule cell output was found to hamper the ability of mice to discriminate even between simple odor stimuli.

The work presented here is the first to evaluate olfactory discrimination behavior after abrogating granule cell output in the olfactory bulb. Unlike the previous studies, where only the rate of granule-cell mediated inhibition was altered to dissect the granule cell function, this is the first study to present the behavioral outcomes of completely abolished the granule cell mediated inhibition on the mitral and tufted cells of the olfactory bulb. The present study also uncovers a previously unclear but fundamental contribution of olfactory bulb granule cells towards optimizing the speed of simple odor pair discrimination.