Abstract

Learning from the surrounding environment can give rise to stable form of memory which helps in interpretation and judgment of the usefulness of the inputs. There is several form of learning. One of the specific form of implicit learning is habituation, where repeated presentation of a stimulus results in a reversible blunting of subsequent behavioral response. Despite its ubiquity and significance, the underlying neural circuit and how it involves synaptic plasticity in the central brain in the process of behavioral habituation are poorly understood. In this thesis, I tried to understand this process with greater mechanistic detail and thereby to propose the neural and molecular mechanism underlying habituation using Drosophila melanogaster.

This study is focussed on olfactory habituation in fruit fly. With the use of two odorants, carbon di-oxide (CO₂) and ethyl butyrate (EB), it was observed that prior odorant exposure results in a selective reduction of response to that odorant. This olfactory habituation happens in two timescale, short term and long term depending upon the duration of odorant exposure. Short term habituation (STH) recovers with a half life of an hour whereas long term habituation (LTH) stays more than a week. Drosophila olfactory circuit is consisted of three kinds of neuron, namely OSNs, LNs and PNs, which synapse onto each other resulting in the formation of the neuropilar structure called glomerulus. Along with the behavioral attenuation, LTH is also associated with glomerulus selective volumetric increase in the antennal lobe. Long term exposure to CO₂ results in V glomerulus volume increase where DM2, DM5, DM6 glomeruli change in volume after exposure to EB. LTH is also observed to be accompanied with odorant selective reduction in PN response. This has been demonstrated by expressing genetically encoded calcium indicator (GCaMP) in EB specific PNs and performing two photon functional imaging to look for odorant-evoked calcium fluxes in PNs. Four days of EB exposure leads to significant reduction in PN response in DM2 and DM5 glomeruli when EB puff was delivered but not in the case when CO₂ delivered. Several experiments with genetic manipulation to perturb different kind of neurotransmitter release from local interneurons and blocking the function of their cognate receptor in PNs indicates that glutamate and GABA release from LN1 subset of local interneurons and Rdl (GABAᴀ receptor) and NMDAR function in odorant selective PNs are necessary to induce
habituation. Glutamate receive and depolarization by the ORNs at the same time make NMDAR (acts as coincident detector) active in PNs and that induce potentiation or strengthening of particular LN- PN synapse resulting in odorant specific habituation.

The unique distinction between short term and long term memory is that, the latter require new protein synthesis during the formation of memory. Synapse-specific plasticity associated with long-term memory can be achieved by local control of synaptic mRNA translation. This study demonstrates the requirement of Ataxin 2 (Atx2), a molecule known to have function in RNA regulation and involved in neurodegenerative disease like spinocerebellar ataxia-2 (SCA2), in presynaptic LN and postsynaptic PN during odorant induced LTH but not for STH. Ataxin 2 knock down in PNs results in selective block of LTH-associated structural and functional plasticity in odorant-responsive glomeruli. Biochemical and cell biological studies indicate that many RNA regulatory proteins like me31b, Argonaute 1 (Ago1) and Ataxin 2 binding protein (A2BP1) bind to Atx2. Transheterozygote studies were performed to find out the trasndominant interaction of Atx2 with these molecules. It was observed that strong dominant genetic interaction between Atx2 and me31b, Ago1 and A2BP1 is necessary for LTH and its associated structural change. These genetic interactions point to a likely role for the Atx2 protein in regulating Ago1- and Me31B-dependent, miRNA mediated translational repression. Several clonal studies indicate that Atx2 is required for optimal repression of several miRNA target mRNAs. Together these observations point to a role for Atx2 in LNs and PNs in miRNA-mediated translational control in the regulation of long-term memory.

In summary our study proposes an underlying neuronal circuit and its modification by induction of olfactory habituation in Drosophila and also point towards a general circuit mechanism for habituation across organisms. This is the first study to distinguish between short term and long term form of habituation in terms of requirement of Atx2 mediated miRNA function and local translation regulation.