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1) Response decorrelation and pattern separation in the dentate gyrus are predominantly mediated by heterogeneities in afferent connectivity

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Behavioral and computational studies implicate adult neurogenesis as a critical component in achieving response decorrelation and pattern separation in the dentate gyrus. To what extent are diverse forms of inherent biological heterogeneities, including those introduced by adult neurogenesis, necessary in accomplishing such input discriminability? We first generated intrinsically heterogeneous populations of conductance-based basket and granule cell models and establish cellular-scale degeneracy in these neurons. We constructed networks of these models, and incrementally added other forms of biological heterogeneities to assess input discriminability under scenarios where these networks received identical or heterogeneous external inputs. Although diverse forms of heterogeneities synergistically contributed to input discriminability, the contributions of local network heterogeneities were strikingly low when the network received heterogeneous afferent inputs. Our results unveil a novel convergence of cellular- and network-scale degeneracy, and question the need for adult neurogenesis towards achieving input discriminability in a divergent network endowed with afferent heterogeneities.

2) Visual and mechanosensory integration by descending interneurons in hawkmoths

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Flying insects use sensory cues from multiple modalities to stabilize their flight. They have to acquire, process and respond to perturbations within a few wing beats to have a stable flight. In the nocturnal hawk moth *Manduca sexta*, mechanosensory input from antenna has been shown to be critical for stable flight. These moths also make use of visual inputs to control their flight. Acquisition and processing of visual cues is typically slower than that of mechanosensory cues, especially so in nocturnal insects because of low light levels. How these sensory cues of different modalities are combined for flight control is not known. For the entire flight control circuit to be fast, we hypothesised that multiple sensory cues are combined in the brain before getting transmitted via a few descending interneurons(dIN) to the thoracic ganglion rather than being sent independently via many dIN. To test this, we recorded intracellularly from axons of dIN in the ventral nerve cord of the nocturnal hawk moth *Daphnis nerii*. We have identified multiple classes of dIN which respond to both mechanical and visual inputs. We have characterized the responses of these different classes of dIN to understand their response to various sensory cues (visual, mechanosensory and combined inputs).

3) Octopamine receptors on Central brain dopaminergic neurons regulate flight durations in *Drosophila melanogaster*

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Insect flight is a complex behaviour that requires the integration of multiple sensory inputs with the motor output. Flight defects can arise from either the inability to sense stimuli, from incorrect information processing in the interneurons or due to malfunctions in the motor output. Here we have studied a catecholaminergic circuit, that helps integrate different sensory cues in the central brain. This circuit sustains longer flight durations, essential for reaching fresh sources of food, finding a mate and identifying suitable places for depositing eggs in the fly's natural environment. Previous studies have shown that *Drosophila* flight can be modulated by monoamines like octopamine, dopamine and serotonin as well as several neuropeptides. Octopamine, analogous to vertebrate norepinephrine, is known to act as a neuromodulator as well as a neurotransmitter in invertebrates. Flies lacking octopamine fly for shorter durations even though their flight muscles appear normal 1. There are approximately 100 octopaminergic neurons in the central nervous system of *Drosophila* that innervate multiple regions of the brain. Earlier we had shown that maintenance of flight for long durations requires the Protocerebral Anterior Medial (PAM) cluster of central dopaminergic neurons 2 . Here we have identified a requirement for specific octopaminergic inputs to the PAM cluster. PAM neurons in turn project to the Mushroom Body (MB), a higher integrating centre in insect brain. Our data support a model where this octopaminergic-dopaminergic circuit integrates sensory information to help generate a contextually relevant flight motor output. In a broader context, this flight circuit functions like the vertebrate circuit where basal ganglia act as an action selection centre based on the inputs received from different regions of the brain, mainly the cortex 3 . Our studies reveal that MB also acts as a context dependent action selection centre like basal ganglia in addition to its learning and memory related functions, which results in a motor output, here this being longer flight durations.

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4) Cx35 encoded gap junctions guide Purkinje neuron dendritic arborization

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The Purkinje neuron (PN) is central to cerebellar function. It receives an astounding number of synaptic inputs from different cells, yet, the connections are always stereotypical and precisely formed. What are the rules governing this pattern formation? How is the precision maintained? We wanted to answer these questions using in vivo imaging of dendrites and electrical and chemical synapses of zebrafish Purkinje neurons. Gap junctions (GJs)/electrical synapses appear prior to

chemical synapses and are thought to direct chemical synapse formation. Gap junctions are composed of Connexin protein subunits (Cx), with Cx35 being a widespread component in zebrafish CNS. Cx35 is expressed by PNs beginning at 4 days post fertilisation (dpf). Using single cell electroporation of neurobiotin, we found that PNs form rectifying GJs with granule cells. To investigate the functional role of Cx35 in PN development, we generated Cx35 $-/-$ zebrafish using TALENs. Transmission electron microscopy of the molecular layer revealed significantly lower number of chemical synapses in Cx35 $-/-$ fish as compared to wildtype fish. Also, single cell patch clamp recordings showed a significantly lower frequency of miniature excitatory post synaptic currents (mEPSCs) in Cx35 $-/-$ PNs, indicating fewer chemical synapses on these neurons. We wanted to test the causality of these results by observing the dynamics of GJs and chemical synapses as PN dendritic arbor develops. For this we undertook in vivo time lapse imaging of PNs in wildtype and Cx35 $-/-$ fish. We labeled single PNs with fluorescent protein markers and imaged them daily from 5dpf to 8dpf. We observed a steady increase in total dendritic branch length (TDBL) and total dendritic branch number (TDBN) from 5-7 dpf. This growth was hampered in Cx35 $-/-$ fish with significant decrease in TDBL observed at 7dpf, as compared to wildtype neurons at the same age. To determine why PN dendritic arbors in Cx35 $-/-$ fish were stunted, we imaged PNs at hourly intervals at 6dpf. This experiment showed that PNs in wildtype and Cx35 $-/-$ fish retracted similar amounts of dendritic lengths but PNs in Cx35 $-/-$ fish have significantly lower addition rates. This result demonstrates that in the absence of Cx35 encoded gap junctions, PNs might receive fewer signals from the surrounding cells and therefore do not add as much as the wildtype neurons to their dendritic arbor. This ultimately results in reduced chemical synaptic density on their arbors. We are currently testing whether electrical synapses might act as passages for cross talk between connected cells or nodes for dendritic stabilization and thus as anchors for chemical synaptic molecules to assemble and form functional contacts.

5) Molecular interactions determining the Regulation of Amyloid Precursor Protein localization at the membrane

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The Amyloid Precursor Protein (APP) is the severely implicated in Alzheimer 's disease (AD). Till date there have been lot of studies which have characterized the role of APP in AD. However there have been very few studies which focus on native function of APP in relevant sub-cellular compartments of neurons. Recent studies from our lab [Kedia et al., unpublished] and other labs [Das et al., 2015] indicate that APP is present in distinct pools across the post-synaptic membrane. This indicates that APP may have a crucial role in functioning of synapses and neural transmission. Among very few known interacting partners of APP identified through literature, we are trying to investigate MINT, PICALM and LRP-1. These interactions are often near the membrane. We have tried to characterize diffusion dynamics of various MINT proteins by single particle tracking, and identified MINT-3 with lowest diffusion dynamics. PICALM is interacts indirectly with APP for endocytosis of APP molecules in endothelial cells. Hence we tried to address surface retention of APP by performing Fluorescence Recovery after Photo-bleaching (FRAP). We observed that, under influence of PICALM recovery of APP-WT and APP-SWE (mutant implicated in AD) is greatly reduced. Similar to these results we expect a difference in APP surface dwell time when LRP-1, MINT interaction of APP is affected. These interactions may be crucial for survival of synapse and hence of higher importance in AD.

6) The expression of fear memories formed before stress is encoded differentially by the amygdala and mPFC

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Exposure to severe stress leads to the development of psychiatric disorders. Clinical studies have shown that three brain areas involved in learning and memory—the hippocampus, amygdala and medial prefrontal cortex (mPFC)—undergo distinct changes with stress disorders. While the hippocampus and mPFC show impairment in structural and functional changes, the amygdala shows an enhancement. Despite these three brain regions having strong anatomical connections, most of these studies focus on individual brain regions. However, recent studies have shown that these connections between regions have strong functional implications. The connectivity between the mPFC and the amygdala has recently been shown to be crucial for fear expression (Likhtik et al., 2014). The effect of stress on the functional connections between these regions is poorly understood. Therefore, we performed in-vivo local field potential recordings from the mPFC and the amygdala in awake behaving rats during fear expression. We found that stress differentially regulates the activity in the mPFC and the amygdala during fear expression. Consistent with cellular findings, the activity in the amygdala is upregulated by stress during fear expression. However, the activity of the mPFC is unaffected by stress during fear expression. We also found that stress causes a decoupling between the activity in the amygdala and mPFC. Interestingly, an earlier study showed that stress strengthens the coupling between the hippocampus and the amygdala (Ghosh et al., 2013). Therefore, although chronic stress impairs structure and function in both the hippocampus and mPFC, the interactions of these two areas and the amygdala appear to be affected in a contrasting fashion. Functional connectivity gets stronger from amygdala to hippocampus but it gets disrupted between mPFC and amygdala. Future studies need to focus on mechanisms involved in these connectivity changes.

7) An Algorithmic Barrier to Neural Circuit Interrogation

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Recent remarkable experimental advances include the ability to perform simultaneous all-optical readout and manipulation of neural activity and the capability to independently control subsets of neurons via, say, two-photon optogenetics in awake behaving animals. Already, such techniques are being used to simultaneously image whole brain activity (e.g. in zebrafish larva). Given this rate of progress, the prospect of understanding exactly how networks of neurons mechanistically perform computations that lead to specific behaviors seems increasingly within reach.

Investigating this type of question will require efficient algorithms for neural circuit interrogation. The algorithms will seek to prescribe the smallest number of experiments necessary (say as a function of the number of neurons in the network), where each experiment may involve neural circuit manipulation, while attempting to elicit the behaviour in question. Also, of fundamental interest, are questions about (computation in) the network that would need a prohibitively large (e.g.

exponential in the number of neurons) number of experiments to determine answers; the answers to such questions are therefore de facto unknowable.

Here, I consider the question of which subset of neurons participate in producing a behavior in a hypothetical experimental setting. Notions of participation are first made precise. I assume that one has the experimental capability to silence arbitrary subsets of neurons and ascertain if the behavior can be elicited. The question, then, is to determine which subset of neurons participate in the said behavior. Using tools from Theoretical Computer Science, I prove, mathematically, that the decision version of this problem is NP-complete and establish algorithmic reductions from this decision problem to the most general version of this problem. This suggests that this problem needs exponentially-many experiments in the number of neurons. Determining which subset of neurons are involved in a behavior is at least as hard, computationally, as understanding how they mechanistically perform computations that lead to the said behavior.

This result implies a strong (and hitherto unexpected) algorithmic barrier to understanding mechanistic computation in neuronal networks and suggests that algorithmic intractability might pose a fundamental roadblock in understanding the brain.

8) Amygdalo-hippocampal connectivity during REM sleep after differential fear conditioning

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Fear memories are formed much rapidly than other memories. What, when and how fear memory is formed not clearly known. In the present study, we show in rats how hippocampus and amygdala gets connected during the rapid eye movement sleep after retrieval of differential fear conditioning. Local field potentials (LFPs) were recorded simultaneously from CA1 hippocampus and Lateral nucleus of amygdala (LA) during sleep in rats. The recordings were carried out within 30 minutes after differential fear conditioning (DFC). The procedure consisted of habituation to the context, differential fear conditioning and retrieval test. Sleep recordings were compared between habituation and retrieval test.

The freezing behavior was used as an index of fear. Results showed DFC increased freezing more specifically to CS+ and less fear to CS-. Further increased freezing behavior during retrieval session was associated with increased REM sleep when compared to that following habituation. The study further showed that the network activity is very different in hippocampus and amygdala during REM sleep. Strong correlation was observed between fear memory and network activity in the amygdala-hippocampal circuit during REM sleep making a strong evidence for the memory consolidation takes place during REM sleep.

Fear conditioning has enhanced REM sleep, with increase in theta power in Lateral Amygdala (LA) and reduced theta power in CA1 hippocampus. But, increased theta power observed in LA during the first 2 hours of sleep was reduced gradually during the later stage of the sleep. In the case of CA1 hippocampus, reduction in theta power was consistent throughout the recording session during REM sleep. This indicates that there is a gradual reduction in fear conditioning-induced theta modulation over the period of sleep. The time scale of this gradual change in LA theta power may vary for the reasons of memory consolidation process.

Keywords:- Differential Fear Conditional, Lateral Amygdala, Hippocampus, REM sleep, Amygdala-hippocampal circuit, Theta power.

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9) Cellular and network level selectivity of spatiotemporal input

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In many forms of brain activity, ensembles of neurons fire sequentially, representing features of sensory stimuli, motor patterns, or internal computations. Hippocampal neurons exhibit sequential activity when animals are traversing a linear track, during replay events, trace conditioning and associative learning [1, 2]. In order to understand neuronal sequence computation for such diverse functional inputs, we study signaling processing at different levels viz, from subcellular levels to network level computations. Here we present modeling studies that focus on the various levels of neuronal computations: A) To study network computation of sequential spatiotemporal input, we have implemented abstract models of neurons with sequence-selective synaptic rules, to show discrimination of spatiotemporal inputs. We are investigating computational implications of the network populated with sequence recognizing neurons to parse various spatiotemporal inputs. B) To show that the projections from sets of stimulus ensembles to compact dendritic zones on single neurons may be common, we analyzed the formation of convergent and ordered projections in random networks representing parameters of hippocampal and cortical networks. C) To examine how sequence computations may take place over multiple time-scales, we have modeled the role of different subcellular mechanisms and their interactions in discrimination of input sequences that vary in time and space. Selectivity of electrical [3] and chemical signaling [4] mechanisms for sequential inputs was previously shown. We are addressing interactions of such subcellular mechanisms along with electrochemical signaling (Calcium Induced Calcium Release) in sequence discrimination using abstract models. Attempts at modeling the cell wide response with detailed multiscale multicompartamental model including subcellular mechanisms and their role in discrimination of spatiotemporal inputs are ongoing. These studies will demonstrate the neuronal computations at single neuronal and network level in discriminating various spatiotemporal input patterns.

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10) Large visual stimuli induce two distinct gamma oscillations in primate visual cortex

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Gamma rhythm (30-70 Hz) has been associated with high level cognitive functions and known to be elicited in many parts of the brain. Gamma oscillations elicited in the primary visual cortex have been extensively studied but its functionality is poorly understood. Although recent reports have

suggested the presence of two gamma oscillations in the hippocampus, only a single gamma rhythm (whose center frequency varies depending on the properties of the visual stimulus) has been observed in the primary visual cortex. We recorded local field potentials (LFP) from chronically implanted microelectrode arrays in the V1 area of two macaque monkeys and found that large visual stimuli that cover both visual hemi-fields induce a slow gamma rhythm between 25-40 Hz in the primary visual cortex of awake monkeys, in addition to the previously reported fast gamma between 45-70 Hz. The tuning preference for stimulus parameters like orientation, contrast and spatial frequency were different for both these rhythms. Further, fast gamma had short latency, strongly entrained spikes and was coherent over short distances, reflecting short-range processing, while slow gamma appeared to reflect long-range processing. Lack of evidence from previous studies in observing two gamma rhythms can be largely attributed to the use of small stimuli localized around the classical receptive field. Two gamma rhythms with different tuning preferences might together provide a more extensive representation of the external input and better coding or communication mechanisms in the primary visual cortex.

11) Biophysical basis of disruption of alpha rhythm in Alzheimer's disease

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Alpha waves are one of the most prominent rhythms (7.5–12.5 Hz) that are detected in electroencephalography (EEG) during wakeful relaxation with closed eyes. In response to changing basal acetylcholine levels, thalamic pacemaker cells orchestrate the alpha rhythm via an intrinsic bursting of a subclass of thalamo-cortical cells (HTC). This rhythm is down-regulated and lower in coherence in Alzheimer's Disease (AD) patients. Furthermore, Acetylcholinesterase (AChE) inhibitor class of drugs that prolong acetylcholine transients seem to provide temporary symptomatic relief in AD. This suggests that the down-regulated alpha rhythm in AD can be a valuable model system to gain a mechanistic understanding of disrupted signaling cascades in the disease. One of the critical components in determining alpha, hyperpolarization activated cyclic nucleotide channels (HCN), is seen to have reduced expression in AD neurons. In a biophysically detailed network model of a thalamo-cortical circuit that generates the alpha-rhythm, we investigate the effect of lowered HCN channel expression. We show that elevated acetylcholine levels (representing action of AChE inhibitors) can compensate for HCN under expression to rescue alpha rhythm. However, increasing acetylcholine levels leads to lowered activation of HCN channels which has been shown to cause increased amyloid-beta aggregation. Our model predicts that slowing down of alpha rhythm due to lowered HCN expression sets up a recursive feedback loop that enhances the inhibitory drive to the HTC. This is consistent with observations of increased levels of GABA in AD tissue. Exaggerated calcium signaling, leading to increased excitability, another key readout of AD tissue, can enhance alpha power. However our results show that it makes the system noisy. Lowered HCN expression can further increase regimes of chaotic behaviour and give rise to an abnormal calcium homeostasis. Our model provides a physiological basis to identify potential network motifs underlying the alpha rhythm that is robust as observed and also responsive to behavioral changes. Modulation in the power of alpha band is seen to characterize distinct behavior. Our results suggest that dynamic changes in phase relationships between HTC cells could explain these rapid transitions in response to changing behavioural states.

12) Nanoscale organization of Amyloid Precursor Protein in an excitatory post-synapse

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The transient composition of the post-synaptic membrane of synapses is controlled by passive diffusion of molecules on the synaptic membrane as well as active processes like endo- and exocytosis. The alteration in number and lateral organization of transmembrane molecules in post-synapse is considered as a crucial factor in health and diseases like Alzheimer's Disease (AD). AD is the most prevalent form of dementia in the elderly. In the last decade a paradigm shift was observed towards understanding the molecular and biochemical pathways implicated in AD where the onset of the disease was proposed to be an altered function of synapses. This resulted in a careful evaluation of the biochemical pathways which regulate the Amyloid Precursor Protein (APP) as well as the development of mouse models for AD. Despite the enormous efforts, the finer mechanisms involved in the early onset of disease still remains unclear. This is partly due to the lack of in-depth evaluation of the mechanisms governing the spatial and temporal evolution of molecular machineries involved in the regulation, retention and recycling of APP. It is already known that genetic alteration of the APP is one of the major causes of Familial Alzheimer's Disease (FAD). Here we try to combine single particle tracking and super resolution imaging to compare the organization and trafficking of wild type APP (APP^{wt}) and a genetic variant of APP (APP^{swe}) identified in FAD. We combined high density single particle tracking techniques like Photo Activation Localization Microscopy (sptPALM) and Direct Stochastic Optical Reconstruction Microscopy (dSTORM) to map trajectories of individual APP molecules on the plasma membrane. Additionally, we illustrate diffusional behaviour from thousands of spatially discrete single molecule trajectories from a single cell with which it is possible to appreciate finer details of versatile molecular mechanisms pertinent in the organization and recycling of APP molecules at the membrane.

13) Brain Gene Expression Changes During Daily Foraging in Honey Bees

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Honey bees have been a successful model system to study diverse learning and memory processes under free flying conditions and in restrained lab assays. Here, we report gene expression changes occurring in the brains of time-trained free flying honey bees.

We trained nectar foragers to an artificial feeder for several days in an outdoor flight cage and collected those at different time points before, during and after the time of feeder presentation. First, honey bees foraging at a known feeder showed a long lasting up-regulation of three immediate early genes (Egr-1, Hr38, kakusei) and also an up-regulation of candidate downstream genes of Egr-1 (EcR, Ddc, DopEcR). Second, we also found that Egr-1 mRNA levels also showed significant up-regulation at the time of feeder training compared to time points before and after feeder training, when we did not present the feeder. Based on this finding, we hypothesized that the Egr-1 expression might be under the regulations of the circadian clock. To test this, we prevented the time-trained foragers from leaving the hive using the artificial rain paradigm. In these experiments, we also observed a significant but lower up-regulation of Egr-1 around the time of feeder training. Together our results indicate two different genomic responses occurring during daily foraging: (a)

continuous daily foraging induces a genomic response mediated by immediate early genes like Egr-1, HR38 and kakusei, and (b) time-training results in an anticipatory up-regulation of Egr-1.

14) Synergy and redundancy in diamond motif : an information-theoretic study

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It has been speculated now for a long time that complex biological systems have meticulously preserved numerous modular structures to promote better adaptability in dynamic system-environment interactions. These special topologies, better known as network motifs, can do numerous articulated physical computations necessary for their sustenance in terms of cell-to-cell communication, harvesting food, antibody resistance, compatibility in sensory-motor neuronal activities etc [1]. To have a better insight into the unifying physical principles [2] encompassing these diversified biological objectives, a physicist turned systems biologist often resorts to a generalised mathematical formalism of information theory which has its origin in communication technology [3, 4]. We select the Diamond Motif (DM) as our model system as it is abundant in different biological and physical settings e.g., in ecological food-chains, electronic logic chips and the synaptic network of *C. Elegans* [1]. We ask why DM is abundant (compared to its functional sub-motifs) in these networks built across length scales and working across time scales? To better understand the delicate working methodologies of DM, we choose the metric of Net Synergy (NS) which originates from neuroscience due to partial information decomposition [5, 6, 7]. NS helps to investigate the complex correlation patterns of DM in terms of Shannon mutual information computed in bits. NS predicts the quantity and quality of information being provided by the nodes of the DM. NS can characterize the nature of information processed by the network e.g., synergistic and redundant information. In some special contexts, information independence may also arise [5]. We have further linked the fidelity measure of DM with NS in the light of evolutionary selection mechanism. The predictions made are experimentally testable in synthetic setup.

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15) Mapping Time to Hippocampal CA1 Sequences

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Small populations of Hippocampal pyramidal neurons from the CA1 are known to take part in reliable, time-locked, activity sequences. In trace memory experiments, we have earlier shown that the interval between the conditioning stimulus (blue LED flash) and the unconditioned stimulus (a puff of air to the eye) is represented by a sequence of activity of these neurons. A subset of these neurons is triggered by the initial stimulus, and then successive subsets become briefly active in succession to bridge the time gap between the two stimuli. We are interested in further understanding these CA1 activity sequences, by asking,

1. How stable are the sequences over time?
2. How does changing the Inter-Stimulus Interval (ISI) affect the population of CA1 neurons that participate in the response?
3. How do responses evolve with habituation and re-learning?

In our experimental design, we train head-fixed mice to a Trace Eye-Blink Conditioning task as described above, where a blue LED flash (CS) is followed after an interval by an air-puff to the eye. We use programmed microcontrollers (Arduinos) to deliver stimuli and synchronize video recording of eye-blinks. The animals that learn the task exhibit conditioned responses such that their eye-blink begins just before the expected air-puff.

To study the activity in the CA1 network, we utilize optical imaging of activity from transgenic mice expressing the calcium reporter GCaMP6f. We image the Ca²⁺ activity using a custom-built two-photon microscope with galvanometric scanning at a frame rate of 10-15 Hz. We monitor the activity of ~100 cells in each field of view, in vivo. In order to monitor the evolution of responses, we perform multi-day recording of activity from the same cells, coupled with the behaviour.

16) Time restricted feeding/foraging and entrainment of circadian clock in honeybees

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Apis mellifera, the European-African honeybee species, has been known for their remarkable time memory and highly adaptive circadian clock. They can virtually learn any time of the day when food is available and adjust their daily activity rhythm accordingly, visiting a food source only when it is most profitable. Like mice and other animals, time restricted foraging results in food anticipatory behavior and entrainment of daily activity rhythm in honeybees too. However, the molecular basis of food entrainment in insects has not been studied much. Also most of the food entrainment experiments are done in constant laboratory conditions and it is not clear whether and how feeding or foraging activity functions as a zeitgeber under natural light-dark cycle. To investigate the effect of restricted foraging time on the molecular clock under natural conditions, I performed a time-restricted feeder training experiments in an outdoor flight chamber (12m x 5m x 2.5m). I trained honeybee foragers to visit an artificial (sucrose solution) feeder either in the morning or in the afternoon for several consecutive days and analyzed the daily clock gene expression rhythm in different brain parts.

I found that the phase of clock genes expression rhythm (Cry2, PER) was significantly different between morning and afternoon trained foragers. More importantly, the phase difference was even larger when the feeder was scented with an odor (linalool).

17) Tunable feedback loop controls airflow mediated antennal positioning in hawkmoths

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The insect antennae are multimodal sensory organs, which sense odor, airflow, humidity, temperature and also mediate flight stabilization. However, their multimodality also means that they must be positioned in a way that allows them to optimally acquire information from various sensory modalities. This task is achieved in insects via dynamic positioning of their antennae, based on information from multiple sensory cues including optic flow and airflow. However, combining multiple cues may be inherently problematic as different modalities process the simultaneous sensory stimuli with different latencies. How do antennae perform this multisensory integration to determine their position?

We began this study by first investigating how antennae integrate airflow information to position themselves. Using experiments and computer simulations, we show that the antennal system breaks down the task of dynamically positioning the antennae into two sub-tasks. One, the computation of position (or set-point) and two, the maintenance of set-point. Thus, by using two sub-circuits for each sub-task, the antennal position can be tuned to respond to perturbations rapidly whilst retaining the ability to use multiple modalities to determine position. This neural architecture is robust and is likely conserved across taxa.

18) Where is my food? Molecular and neuronal correlates of nutrient sensing in Drosophila larvae

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Organisms need nutrients to generate energy for various physiological processes and therefore possess mechanisms to sense fluctuations in external nutrient levels. *Drosophila* have two predominant stages in life where they are dependent on external nutrients viz. the larval and the adult. Third instar larvae feed voraciously and when an optimal internal metabolic state is achieved, pupariate. However, often in ethologically relevant scenarios, these organisms find themselves limited by resources and then the decision to pupariate requires integrating the environmental information with the best possible internal state. This integration is performed by neurons in combination with signalling pathways. IP3R and the associated Store-operated calcium entry (SOCE) are signalling processes that help transform external stimuli into elevated cytosolic calcium levels and meaningful physiological responses. We have previously reported that IP3R/Ca²⁺ signalling in glutamatergic interneurons in *Drosophila* larvae enable response to an environment lacking amino acids for pupariation. Here, we report that calcium transients in glutamatergic

neurons encode withdrawal of amino acids. These calcium transients are dependent on neuronal activity regulated by IP₃R and SOCE. We also report that the withdrawal of one such essential amino acid, arginine, is sensed by class IV multidendritic ppk neurons. Inputs from these multidendritic neurons drive activity in glutamatergic neurons for integrating nutrient information. Activity in this network is important in larvae for making active decisions pertaining to preferential diets.

19) Tight balance controls gain and timing in hippocampus

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Neural networks typically require a balance between excitation and inhibition. Theoretical studies have predicted that for temporally coding networks, all excitatory inputs to a neuron should be followed by proportional inhibitory input, on a millisecond timescale. This phenomenon is known as tight balance. Since CA1 neurons are known to do temporal coding, we asked if CA3 inputs to CA1 (largest input stream to CA1), display tight balance.

To address this, we performed patterned optical stimulation of channelrhodopsin2-expressing CA3 neurons in acute hippocampal slices, and recorded from voltage clamped CA1 neurons. We presented each cell with up to 30 different stimuli, which were random combinations of 1 to 9, 16µm x 16µm spots. We find that for a given CA1 cell, any randomly selected subset of CA3 neurons evokes proportional excitatory and feed-forward inhibitory responses, confirming the existence of tight balance in the CA3-CA1 network.

We next asked how these tightly balanced inputs integrate at the CA1 soma. In order to explore a wide dynamic range of inputs, we presented > 100 combinations of 1, 2, 3, 5, 7, or 9 input spots/stimuli to each cell. These stimuli typically elicited subthreshold post-synaptic potentials (PSPs). Over the large range of inputs, the PSPs followed a divisive normalization curve. Of the 3 different models - divisive normalization, divisive inhibition and subtractive inhibition - we tested on our data, divisive normalization had the best fit. This Subthreshold Divisive Normalization (SDN) is a novel form of gain control, which expands the number of inputs a single neuron can accommodate before it reaches spike threshold. Using a computational model we predicted that mechanistically, SDN requires exquisite balance along with a specific relationship of E-I latency. Our data confirmed the prediction that E-I latency is inversely proportional to excitatory conductance.

While SDN allows integration of a large range of inputs, at high input amplitudes, it results in saturation of PSP peaks. The accompanying saturation implies loss of information of input amplitudes. Interestingly, we find that this information is not lost, but simply transferred from amplitude to time of peak of the PSP, with larger number of inputs leading to earlier time to peak. This is a way by which spike timing can be controlled at millisecond time scales, which is relevant to phenomenon like hippocampal replay of memories (Jadhav, 2011).

To summarize, we have found that tightly balanced feed-forward inhibition performs Subthreshold Divisive Normalization (SDN), and controls the peak timing of PSPs in the hippocampal CA3-CA1 circuit.

20) Profiling Dopamine as a ligand at the 5HT2A receptor

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Serotonin is an important neurotransmitter in vertebrates and disruption of the serotonergic system has been implicated in various psychiatric disorders such as schizophrenia, bipolar disorder, autism spectrum disorder, etc. Most of the drugs targeting these neurotransmitter systems are classified primarily as agonists or antagonists, with their function described as being limited to setting up the canonical signalling pathway(s), or stopping the receptor from setting up its canonical signalling pathway(s) respectively. Functional selectivity is defined as the ability of a ligand to bias a receptor to occupy different conformations, potentially leading to different signalling pathways being activated downstream.

Previous work with the human 5-HT2A receptor has shown that this receptor can also be activated by dopamine, an endogenous ligand which is part of the dopaminergic system. This system greatly overlaps with the serotonergic system in its distribution in the brain. We have investigated the functional selectivity shown by the 5-HT2A receptor and have profiled dopamine as a ligand at this receptor. To investigate this, we have used transferrin co-localization assays, intracellular calcium rise, and formation of stress fibers as indicators of differences in signalling pathways that are set up by the receptor. We have also used different 5-HT2A mutants to determine the residues important for this functional selectivity shown by the receptor. Our work with functional selectivity of the 5-HT2A receptor could have far reaching implications for the field of GPCR signalling and drug-design.

21) Mechanisms of olfactory divergence leading to sympatric speciation in apple flies (*Rhagoletis pomonella*)

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Host shifting in insects is a well-known mechanism of speciation, but little is known about the neurophysiological phenotypes that trigger these shifts. The apple maggot fly, *Rhagoletis pomonella* is a model for ecological speciation via host plant shifting. Within the past 180 years, flies infesting hawthorn fruit have shifted to attack apple. Today apple race flies positively orient to the volatiles from apples and are deterred by hawthorn volatiles, with the reverse for hawthorn race flies. To understand how neurophysiological pathways contribute to shifts in olfactory preference, we characterized fly neurophysiology on multiple levels. On the antennae of *Rhagoletis* we examined the morphology, specificity, and distribution of olfactory sensory neurons (OSNs). Although the numbers and sensitivities of OSNs were the same between the host races, we discovered the existence of co-localized pairs of OSNs, with one neuron responding to the key volatile from the apple blend and the other to the key volatile of the hawthorn blend. Using a combination of immunohistochemistry, 3-D reconstruction, and optical imaging, we are testing the hypothesis that a simple reversal in the response of these OSNs could account for the behavioral difference between races. Our studies suggest that small changes in olfactory channels can have great impacts on behavior, potentially even influencing speciation events.

22) Homeostatic synaptic scaling in hippocampal neurons is locally regulated

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Synaptic scaling is a form of homeostatic synaptic plasticity, wherein neurons are purported to globally scale all synaptic weights up or down, in the appropriate direction, in order to maintain a stable firing rate. Whether synaptic scaling is regulated globally or locally, and the pre- and postsynaptic mechanisms involved, are still unknown. We report that in cultured hippocampal neurons, only a fraction of synapses undergoes scaling up in response to chronic network activity blockade, suggesting a local regulation of scaling. We also identify a previously unreported role for presynaptic scaffolding protein Bassoon in synaptic scaling. Quantitative immunocytochemistry was used to study the effect of synaptic scaling on several pre- and postsynaptic molecules. The change in the distribution of synaptic area and molecules indicated that there is a subset of synapses, which show a major increase in size and AMPA receptor accumulation during scaling. These results point out that even though the activity deprivation is global, scaling is locally regulated. Future investigations will focus on the differential effects on synapses showing scaling. The role of presynaptic molecules in scaling is another interesting aspect to be explored in detail.

23) Phospholipase D regulates synaptic vesicle recycling

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Phospholipids play a key role in mediating signal transduction events and intracellular membrane trafficking. Phosphatidic acid (PA) is one such phospholipid. PA can be generated in cells by a number of different enzymes, one of which is via the activity of Phospholipase D (PLD). PLD hydrolyses phosphatidylcholine (PC) to generate membrane-bound PA and choline. Overexpression of PLD1 in a range of neuronal cell type suggests PLD can regulate vesicular transport. However the mechanism underlying the role of PLD in regulating membrane transport has remained unclear.

We have recently shown that in *Drosophila* photoreceptors the light sensitive apical plasma membrane (rhabdomere) undergo rapid turnover during illumination resulting in changes in rhabdomere size. This is accompanied by changes in the level and localization of apical membrane protein rhodopsin1. *Drosophila* photoreceptors contain a light induced dPLD activity and during illumination it regulates membrane turnover in an ARF1 and retromer complex dependent manner, thereby aiding recycling of rhodopsin1 containing membrane to the plasma membrane(Thakur et al., 2016). Thus, our studies in *Drosophila* photoreceptors implicate dPLD as a regulator of apical membrane recycling in polarized cells.

We are interested to understand whether dPLD is a general regulator of other membrane trafficking events. To test this, we are studying the function of dPLD in the synapse of *Drosophila* larval neuromuscular junction (NMJ). We find that dPLD activity is required in the neuron but not muscle for normal NMJ morphology and organization. Electron microscopy analysis shows that in absence of dPLD the length of active zone and the number of synaptic vesicles at the active zone is significantly increased. We also show that in absence of dPLD uptake of lipophilic dye FM4-64 is

not affected however post-loading, dye clearance during stimulation is delayed. Overall we find dPLD to be a novel regulator of synaptic vesicle cycling at the NMJ.

24) Spatially dispersed synapses yield sharply-tuned place cell responses through dendritic spike initiation

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A prominent hypothesis spanning several sensory-perceptual systems implicates spatially clustered synapses in the generation of dendritic spikes that mediate sharply-tuned neuronal responses to input features. In this conductance-based morphologically-precise computational study, we tested this hypothesis by systematically analyzing the impact of distinct synaptic and channel localization profiles on sharpness of spatial tuning in hippocampal pyramidal neurons. We found that the generation of dendritic spikes, the emergence of an excitatory ramp in somatic voltage responses and sharp tuning of place-cell responses were all attainable even when iso-feature synapses are randomly dispersed across the dendritic arbor. Strikingly, the generation and propagation of dendritic spikes reliant on dendritic sodium channels and N-methyl-D-aspartate receptors mediated the sharpness of spatial tuning achieved with dispersed synaptic localization. To ensure that our results were not artifacts of narrow parametric choices, we confirmed our results with independent multi-parametric stochastic search algorithms spanning thousands of unique models for each synaptic localization scenario. Next, employing virtual knockout models, we demonstrated a vital role for dendritically expressed voltage-gated ion channels, especially the transient potassium channels, in maintaining sharpness of place-cell tuning. Finally and importantly, we established that synaptic potentiation targeted to afferents from one specific place field was sufficient to effectuate place-field selectivity even when intrinsically disparate neurons received randomly dispersed afferents from multiple place-field locations. Our results provide clear lines of quantitative evidence that spatial clustering of synapses is neither essential for the generation of dendritic spikes nor a requirement for sharp tuning of neuronal responses to input features.

25) Conserved mechanosensory-motor circuits in insect antennae

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Insect antennae are not just olfactory organs, but also serve critical mechanosensory roles in all insects. The variety of morphological forms of insect antennae reflects the diversity of antennal mechanosensory functions, which range from tactile sensation to audition and also, in mediating rapid flight control. The importance of antennal feedback is evident from the precise and maintained positioning of the antennae during flight in nearly all insects. The ubiquity of this so-called “antennal positioning response” in insects suggests the hypothesis that this important behavior may

be conserved despite the vast diversity of insect species. To address this hypothesis, it is therefore essential to probe these circuits using a comparative approach. We investigated this question using

two approaches. First, we characterized the architecture and neural targets of mechanosensory neurons which underlie the hair plates (or Böhm's bristles) located in basal segments of the insect antennae. Second, we surveyed the hair plates and the Johnston's organs, located between the pedicel and flagellum, and asked how conserved these structures are across insect taxa. We chose phylogenetically distinct insect orders, including moths (Order - Lepidoptera), honey bees (Order - Hymenoptera) and crickets (Order- Orthoptera), all of which use antennae in distinct ways. We examined the neural circuits underlying mechanosensors, and observed that these too were conserved. Despite their ecologically, morphologically and behaviorally diverse roles, the axonal projection patterns of Böhm's bristles and their sensorimotor targets within the Antennal Mechanosensory and Motor Centre (AMMC) remain essentially similar, suggesting that "antennal positioning behavior" is an essential feature of flying insects.

26) Investigating the role of STIM1 and Store-Operated Calcium Entry in Mouse Purkinje Neurons

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Calcium ions are a universal second messenger that regulate various processes, such as proliferation, gene transcription, contraction, exocytosis, apoptosis, the immune response, and neurotransmission. Store-Operated Calcium Entry (SOCE) is a major mechanism for calcium mobilization in many non-excitabile and some excitable cells. Store-operated channels (SOCs) open in response to depletion of calcium in the endoplasmic reticulum (ER) after stimulation of cell surface receptors coupled to G-proteins and tyrosine kinases. Store-operated channels (SOCs) consists of the pore-forming Orai proteins that are activated by the ER Calcium sensor STIM (stromal interaction molecule). In the mature brain, store-operated Ca²⁺ entry (SOCE) is thought to be required for the maintenance of neuronal calcium homeostasis, which in turn can influence synaptic transmission and plasticity. However, the status of SOCE through STIM/Orai activation and its relevance to mammalian cerebellar neuron function are not well studied. Studies have proposed that deranged calcium signalling cascade in cerebellar Purkinje neurons might leads to neuronal degeneration and Spinocerebellar Ataxia (SCA). We have used Mouse Purkinje neurons as a mammalian model system to understand how STIM1 modulate neuronal function and how altered function of STIM1 leads to neurodegeneration. In this poster, I will show some of the preliminary work done in wild and STIM1 knock out murine model system. I will share experiments standardised to measure calcium responses in cultured purkinje neurons and also present some preliminary data on behavioral assays standardised to test the motor learning and coordination in mice.

27) Cerebellar Control of Swimming in Larval Zebrafish

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Locomotion is a highly complex task that requires precise co-ordination of the muscles involved. Movement commands generated by the brain lead to a lot of errors when the movement is actually made. These errors are corrected for by the cerebellum and associated regions in the brain. In order to perform this correction, the cerebellum is thought to monitor the actual state of movement and

compare it to the desired state of movement. This comparison results in an error signal that is used to perform compensatory movements to correct for these errors. It is not clearly understood how this correction takes place during locomotion.

Zebrafish larvae exhibit an innate behavioral response, where they stabilize themselves against involuntary motion caused by flowing water. This response can be evoked reliably using visual stimuli that drift one way or the other, across a wide field of view, to simulate flow (optomotor response). To study the neural control of this behavior, we have developed a virtual environment for restrained zebrafish larvae to perform the optomotor response and receive realistic sensory feedback corresponding to any movement they make. This setup allows us to manipulate how motor output of the fish maps to the corresponding sensory feedback (feedback gain) and simultaneously record activity in brain regions of interest during behavior. Preliminary results show motor-locked parallel fiber activity that can be reliably predicted by swim velocity.

28) Interventions after stress to prevent its delayed effects on the amygdala

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A single, traumatic exposure to stress can lead to a delayed emergence of effects, as in Post-Traumatic Stress Disorder. Using an acute stress model in rats, we found that exposure to a single bout (2 hours) of immobilisation stress causes an increase in anxiety (Anxiety Index, Control: 0.51 ± 0.02 , Acute Stress: 0.62 ± 0.03) and dendritic spines in basolateral amygdala neurons (Control: 85.6 ± 2.7 , Acute Stress: 102.4 ± 4.5) 10 days later, but not 1 day later (Mitra et al, 2005). This suggests that the effects develop over time, hence prompting the question if these delayed effects could be prevented by intervening after stress exposure. Indeed, intervention with the anxiolytic diazepam (DZP) 1 hour after acute stress prevented both increase in spines (Control + DZP: 61.8 ± 4.1 , Acute Stress + DZP: 58.2 ± 2.7) as well as increase in anxiety (Anxiety Index, Control + DZP: 0.49 ± 0.04 , Acute Stress + DZP: 0.46 ± 0.03) 10 days after stress. Interestingly, intervention up to 1 day after stress was also sufficient in preventing the delayed effects (Dendritic Spines, Control + DZP: 60.4 ± 4.2 , Acute Stress + DZP: 52.7 ± 3.7 ; Anxiety Index, Control + DZP: 0.51 ± 0.05 , Acute Stress + DZP: 0.52 ± 0.04). Moreover, we also found that intervention with vehicle (VEH) after stress, both 1 hour and 1 day later, was sufficient by itself in preventing the delayed increase in dendritic spines and anxiety (1 Hour: Dendritic Spines, Control + VEH: 72.4 ± 2.7 , Acute Stress + VEH: 65.6 ± 3.8 ; Anxiety Index, Control + VEH: 0.54 ± 0.04 , Acute Stress + VEH: 0.52 ± 0.02 ; 1 Day: Dendritic Spines, Control + VEH: 70.1 ± 4.2 , Acute Stress + VEH: 64.8 ± 3.4 ; Anxiety Index, Control + VEH: 0.52 ± 0.04 , Acute Stress + VEH: 0.52 ± 0.04). The mechanism of this vehicle-mediated prevention is currently being examined to better understand the possible approaches that could eventually translate to therapeutic intervention strategies.

29) Double whammy of stress: Neurons vs. Astrocytes, Hippocampus vs. Amygdala

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Stress leads to contrasting effects on the structural remodelling of neurons in the hippocampus and the amygdala. These structural changes in the neurons strongly correlate to the region specific physiological and behavioural deficits observed in stressed subjects. However, our understanding of cellular correlates of stress is far from complete as relatively little is known about stress induced structural plasticity of astrocytes. Hence, we examined if the contrasting effects of stress extend to the distribution and structural remodelling of astrocytes in these two brain regions. Chronic immobilisation stress (CIS) leads to a reduction in the number of GFAP positive astrocytes in the Basolateral amygdala (BLA) but not in the dorsal hippocampus CA3 (dCA3) region. Using intracellularly dye filled astrocytes, we observe that Individual astrocytes in both Basal (BA) and lateral amygdala (LA) undergo a reduction in the neuropil volume they occupy whereas the astrocytes in the dCA3 do not undergo any such change with CIS. Interestingly, a comprehensive analysis and quantification of the arborisation profiles of these highly ramified protoplasmic astrocytes reveal that CIS leads to subtle differences in the structural reorganisation of the Basal and lateral amygdalar astrocyte branches. The distal branches of astrocytes in LA undergo atrophy whereas the proximal branches of astrocytes in BA undergo hypertrophy with CIS. On the other hand, no changes are observed in the arborisation profiles of dCA3 astrocytes with CIS. Our study points out that the contrasting effects of CIS not only extend to the astrocyte structure and distribution but the direction of astrocytic structural reorganisation is qualitatively opposite to that seen in the neurons of these two brain regions.

30) What happens after a single episode of stress? Changes in neuronal, astrocytic and microglial function over time

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Acute Immobilisation Stress (AIS) is a paradigm to study the effects of exposure to a single episode of life-threatening experience. This paradigm can model the Post-Traumatic Stress Disorder (PTSD). Previous data from our lab and others have shown that there are delayed morphological and electrophysiological changes in the neurons of the basolateral amygdala (BLA) along with an enhanced anxiety phenotype. Furthermore, data also suggests that BLA, and dorsal hippocampus (dCA1) are key brain regions for the manifestation of these changes that are well established to be protein synthesis dependent. However, there has been no focused study on the extent and cell-type specificity of how changes in protein synthesis develop over time. The results presented here are a characterisation of spatio-temporal changes in translation in neurons and astrocytes after AIS. Further, we also dwell into the activation profiles of microglia after AIS.

31) "Socialist" Model of Stress and Emotion: the good, the bad, and the ugly

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32) Characterisation of human pluripotent stem cell-derived neurons from Fragile-X patients

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Fragile X Syndrome (FXS) is the most common form of inherited mental retardation. It is a neurodevelopment disorder caused by repeated CGG repeat mutation in the Fmr1 gene, thereby silencing it. Pre-clinical studies on Fmr1 KO mouse models have shown impact of the loss of this gene on various parts of the brain and the involvement of the a class of glutamate receptors called the metabotropic glutamate receptors (mGluR), but clinical drug trials based on these pre-clinical data have not led to positive outcomes thereby making the need to study this disease in more human-relevant models paramount. We have attempted to study this disease in cortical neurons derived from the fibroblast culture of the patients suffering from Fragile X Syndrome. We have analyzed the functional properties of the neurons using the patch-clamp technique, and we have categorized these into three categories: - a) Intrinsic (Passive electrical) properties, b) Burst properties, c) Synaptic Properties. Preliminary data shows that there is no significant difference in the intrinsic properties of the FXS neurons when compared to the control neurons, which means that the absence of the Fragile X protein doesn't interfere with the basic electrical properties of the human pluripotent stem cell derived neurons. On analysis of the burst properties we find differences in the bursting activity of the FXS neurons and the control neurons. The FXS neurons fire an increased number of bursts in a particular time frame than the control neurons. These bursts of the FXS neurons are lesser in duration than the control neurons, thereby indicating that the loss of FXS protein might mess up the network activity of the neurons. We have also shown some very preliminary data on the effect of an mGluR agonist, on these neurons.

33) Do NLGN3 and Fmr1 KO rats share a common pathophysiology?

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34) mGluR mediated synaptic plasticity in the Lateral Amygdala in a rat model of Fragile X Syndrome

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Metabotropic glutamate receptor (mGluR) dependent synaptic plasticity has been a major focus in studies aimed at understanding the cellular basis of cognitive deficits seen in Fragile X Syndrome (FXS), a common heritable form of mental impairment. However, the majority of earlier studies have primarily examined the hippocampus and the cortex in this context. Despite emotional dysfunctions being a major symptom of FXS, relatively little is known about how the amygdala, a key structure mediating emotional salience, is affected in FXS. Previous studies have shown that the direction of mGluR-dependent change in synaptic strength (i.e. LTP versus LTD) and its aberration (impairment versus enhancement) in Fmr1 KO mice are opposite in the amygdala compared to the hippocampus (Suvrathan et. al. 2010, Suvrathan and Chattarji 2011). The goal of the present study is to understand the synaptic basis of this striking difference.

Pharmacological activation of mGluRs with 3,5-Dihydroxyphenylglycine (DHPG), a potent agonist of group I metabotropic glutamate receptors, manifests as mGluR-LTD in the CA1 region of the hippocampus (Huber et. al.2000). In contrast, the same bath application of DHPG in the amygdala strengthens synaptic transmission in principal neurons of the lateral amygdala. This is manifested as an increase in the frequency, but not the amplitude, of mEPSCs. Despite these striking differences, bath application of DHPG leads to the internalization of surface AMPA receptors in both the amygdala and the hippocampus. This reduction in AMPA receptor surface expression appears to be a common end-point underlying the aberrations in mGluR-dependent synaptic plasticity across brain regions. Moreover, this highlights the need to look beyond postsynaptic deficits and focus on presynaptic mechanisms of mGluR plasticity in the amygdala. Using whole-cell patch-clamp recordings from excitatory projection neurons in the lateral amygdala, we studied the presynaptic effects of mGluR activation and how these effects are altered in a rat model of the FXS. We find that while Fmr1-KO rats have a lower baseline frequency of mEPSCs compared to wild-type rats, DHPG increases the frequency, but not the amplitude, of mEPSCs in projection neurons of the LA. Further confirmation of this comes from studying pair-pulse facilitation at the thalamic and cortical inputs of the amygdala. Together these changes are opposite to the decrease in frequency of mEPSCs seen in the hippocampus (Snyder et. al. 2001).

Finally, we have also explored the functional consequences of this novel form of mGluR plasticity in the amygdala. First, we examined the behavioural implications of mGluR activation in terms of cue-specific and generalized fear. We see that while mGluR activation through infusion of DHPG into the amygdala alone leads to generalized fear, DHPG infusion coupled with fear

conditioning enhances cue-specific fear memory. Second, this entire framework from synaptic plasticity to fear learning is also being utilized to compare wild-type vs.Fmr1-KO rats. In conclusion, our findings provide a novel intellectual framework for mGluR plasticity in the amygdala spanning molecular, synaptic and behavioural mechanisms and lead to valuable mechanistic insights in to the emotional symptoms of FXS.