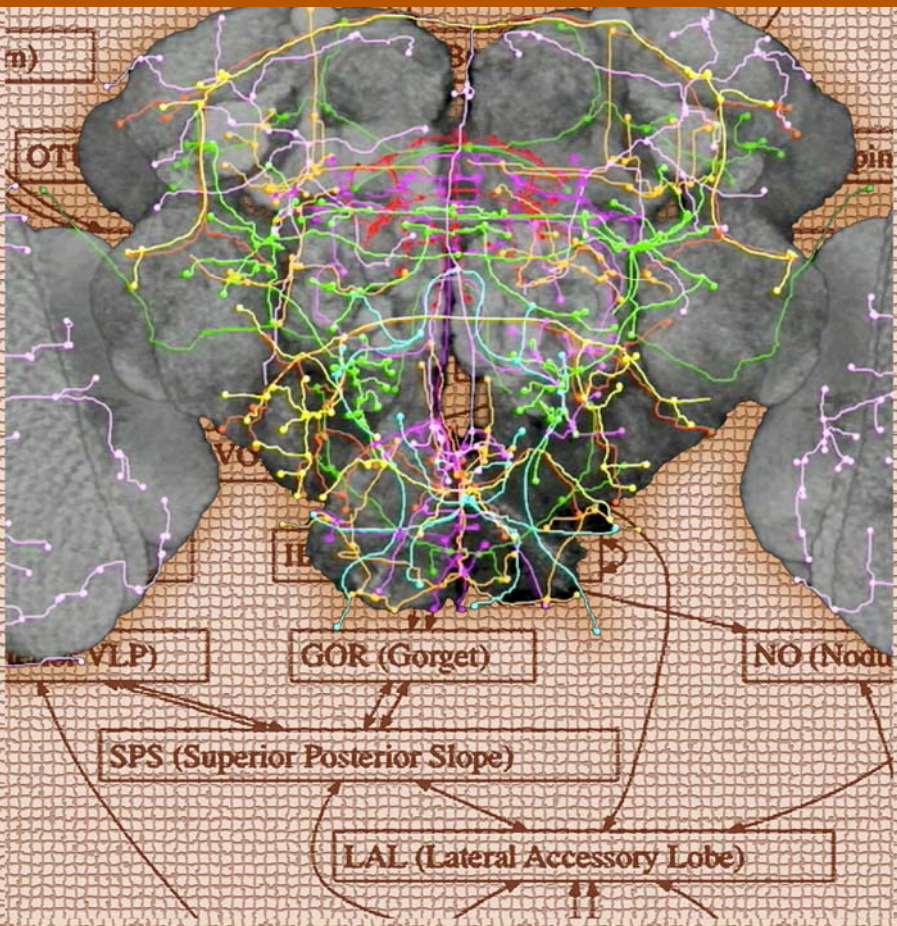
Abstracts of papers presented
at the 2010 meeting on

NEURONAL CIRCUITS

March 10–March 13, 2010



Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

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at the 2010 meeting on

NEURONAL CIRCUITS

March 10–March 13, 2010

Arranged by

Cori Bargmann, *HHMI, The Rockefeller University*
Ed Callaway, *Salk Institute for Biological Studies*
Dmitri Chklovskii, *HHMI Janiela Farm Research Campus*

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Front Cover: A compartment-level 3D neurite wiring atlas of a fruit fly brain, picture produced by Hanchuan Peng with help of Yan Zhuang.

Back Cover: Electron microscopy reconstruction of the *Drosophila* medulla column by Takemura, S., Lu, Z., Vitaladevuni, S., Scheffer, L., Meinertzhagen, I.A., and Chklovskii, D.B.

NEURONAL CIRCUITS

Wednesday, March 10– Saturday, March 13, 2010

Wednesday	7:30 pm	**1** Circuits and Behavior
Thursday	9:00 am	**2** Connectomics / Vision
Thursday	2:00 pm	**3** Poster Session
Thursday	4:30 pm	Wine and Cheese Party
Thursday	7:30 pm	**4** Cortex and Other Circuits
Friday	9:00 am	**5** Methods / Chemosensation
Friday	2:00 pm	**6** Chemosensation / Motor
Friday	6:00 pm	Banquet
Saturday	9:00 am	**7** Larry Katz Memorial Lecture / Neural Computations

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Mealtimes at Blackford Hall are as follows:

Breakfast 7:30 am-9:00 am
Lunch 11:30 am-1:30 pm
Dinner 5:30 pm-7:00 pm

Bar is open from 5:00 pm until late

Printed on 100% recycled paper.

PROGRAM

WEDNESDAY, March 10—7:30 PM

SESSION 1 CIRCUITS AND BEHAVIOR

Chairperson: **L. Luo,** Stanford University, California

SESSION 2 CONNECTOMICS / VISION

Chairperson: **D. Chklovskii,** HHMI Janiela Farm Research Campus, Ashburn, Virginia

THURSDAY, March 11—2:00 PM

SESSION 3 POSTER SESSION

THURSDAY, March 11—4:30 PM

Wine and Cheese Party

SESSION 4 CORTEX AND OTHER CIRCUITS

Chairperson: **E.M. Callaway,** Salk Institute for Biological Studies, La Jolla, California

FRIDAY, March 12—9:00 AM

SESSION 5 METHODS / CHEMOSENSATION

Chairperson: **S. Lockery,** University of Oregon, Eugene

FRIDAY, March 12—2:00 PM

SESSION 6 CHEMOSENSATION / MOTOR

Chairperson: **C. Bargmann,** The Rockefeller University, New York, New York

FRIDAY, March 12

BANQUET

Cocktails 6:00 PM Dinner 6:45 PM

SESSION 7 NEURAL COMPUTATIONS

Chairperson: **M.-B. Moser,** Norwegian University of Science and Technology, Trondheim

LARRY KATZ MEMORIAL LECTURE

Neural codes for spatial representation in entorhinal cortex and hippocampus

May-Britt Moser

NTNU, Kavli Inst./CBM, MTFS, Trondheim, N-7489, Norway

Cognition reflects computations in widespread and entangled neural circuits but the development of these circuits and the nature of the computations are poorly understood. My talk will address two questions. First, I will ask if the brain's spatial representation system is innate. Spatial representation is created in grid cells and other dedicated cell types in networks of the entorhinal cortex and the hippocampus, many synapses away from the sensory cortices. Grid cells fire selectively at regularly spaced positions in the environment such that, for each cell, activity is observed only when the animal is at places that together define a repeating triangular pattern tiling the entire environment, almost like the cross points of graph paper, but with an equilateral triangle as the repeating unit. Grid cells co-localize with other entorhinal cell types such as head-direction cells, conjunctive grid×head direction cells, and border cells, which each contribute to representation of current location in moving animals. I will show that rats that explore the environment outside the nest for the first times have both place cells, grid cells and head direction cells; however, the spatial map is not very stable during the first few weeks after eye opening. Sharply modulated head direction cells are present on the day after eye opening whereas the spatial firing properties of place and grid cells continue to evolve from their initial rudimentary form. In the second part of my talk, I shall examine the mechanisms for retrieval of multiple representations during the same event. I will show that abrupt transformation of one familiar environment into another is often followed by transient flickering between representations for those environments, with different representations expressed on different theta cycles. Mixed-state cycles do exist but are less frequent than expected by chance. These observations point to theta cycles as minimum units of hippocampal spatial representation and imply that individual cycles, but not necessarily successive cycles, tend to remain within one state. They also imply theta cycles as possible units for separation of current information from grid cells and past information from selected place cells.

Dissecting a neural circuit regulating food-seeking behavior in *C.elegans*

Sreekanth H Chalasani[1], Saul Kato[2], Dirk Albrecht[2], Cori I Bargmann[2]

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Neural circuits transform sensory signals to generate behaviors on timescales from seconds to hours. In some *C.elegans* behaviors, sensory inputs lead to long-lasting and complex behavioral sequences. Animals that have been recently removed from food spend about 15 min exploring a local area by interrupting long forward movements with random turns and then disperse by suppressing turning. A pair of olfactory neurons called AWC, regulate the turning probability upon removal from food. The AWC sensory neurons synapse onto many interneurons including AIB which stimulate turns and AIY and AIA that inhibit turns (Wakabayashi *et al* 2004, Gray *et al* 2005).

We have found that AWC sensory neurons become active in response to removal of stimulus releasing two neurotransmitters (glutamate and a neuropeptide NLP-1). AWC released glutamate activates AIB and inhibits AIY and AIA interneurons, promoting turns (Chalasani *et al* 2007). In contrast to glutamate, AWC released NLP-1 acts on AIA interneurons to suppress reversals, suggesting that turn frequencies are regulated by at least two opposing signaling systems. AWC calcium responses are modulated in these neurotransmitter mutants, suggesting multiple pathways can influence AWC neuronal activity and AWC-regulated behavior.

References:

Chalasani, S. H., *et al* (2007). Dissecting a circuit for olfactory behaviour in *Caenorhabditis elegans*. Nature 450, 63-70.

Gray, J.M., *et al* (2005). A circuit for navigation in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. 102, 3184-3191.

Wakabayashi, T., *et al* (2004). Neurons regulating the duration of forward locomotion in *Caenorhabditis elegans*. Neurosci. Res. 50, 103-111.

PRESYNAPTIC FACILITATION BY NEUROPEPTIDE SIGNALING MEDIATES STARVATION-DEPENDENT OLFACTORY BEHAVIOR

Cory M Root, Kang Ko, Amir Jafari, Jing W Wang

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Early sensory processing plays a critical role in detecting environmental cues. In previous work, we found that gain control by presynaptic inhibition of sensory transmission is important for pheromone-mediated mate localization in *Drosophila*. Given that synaptic modulation can improve odor-object localization, it is likely that sensory processing could be enhanced to improve behavior to match the internal state of an animal. We have therefore investigated the effect of hunger on food finding behavior and odor-evoked activity in the first olfactory relay in *Drosophila*. Using two-photon calcium imaging we found that starvation alters olfactory representation in the second order neurons of the antennal lobe by enhancing activity in select glomeruli. Furthermore, we developed an odor-guided food localization assay and found that starvation increases the efficacy of food finding. We next wanted to elucidate the mechanism by which starvation affects olfactory processing and odor-guided behavior. The neuropeptide sNPF, a homolog of the mammalian NPY, is highly implicated in hunger signaling and is expressed in *Drosophila* olfactory sensory neurons (OSNs). We therefore asked if sNPF signaling in OSNs is responsible for the starvation-dependent enhancement of food finding behavior and olfactory processing. Using RNAi to knockdown sNPF expression selectively in sensory neurons, we found that sNPF signaling is necessary for the starvation-dependent modulation of both olfactory representation and food finding behavior. Furthermore, the receptor for sNPF is expressed in OSNs and mediates a feedback enhancement of sensory transmission in select glomeruli. Thus, hunger causes presynaptic facilitation of sensory transmission, which leads to enhancement of odor-guided food finding behavior.

Cell-type specific modulation of visual responses by behavioral state in mouse cortex

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University of California - San Francisco, Dept of Physiology, 513 Parnassus Ave, San Francisco, CA, 94143

Nearly all studies of visual processing in rodents have been performed in anesthetized animals, precluding any examination of the effects of behavior on visually-evoked responses. We therefore performed extracellular multisite electrode recordings in awake, head-fixed animals that were allowed to run on a freely rotating trackball. By measuring the velocity of the ball, we were able to compare responses to visual stimuli when the animal was stationary on the ball versus running. We found a dramatic shift in cortical processing as the animal spontaneously shifts from sitting still to running. In the local field potential (LFP), we observed a general shift from low frequencies to high frequencies, particularly in a narrow gamma band around 50-70Hz. In single-unit recordings, the majority of neurons in V1 showed more than a doubling of response amplitude when the animal was running. Interestingly, neither spontaneous activity nor orientation tuning width demonstrated any consistent change, suggesting that this increased responsiveness is the result of a gain modulation.

Narrow- and broad-spiking cells generally responded similarly to behavioral state change. However, a small subset of narrow-spiking cells dramatically increased their spontaneous rate during running, and their firing rate was then suppressed by presentation of a visual stimulus within the receptive field. Furthermore, in contrast to the increased response observed in visual cortex, neurons in the lateral geniculate nucleus of thalamus showed no consistent change in response amplitude. However, we did observe a decrease in the frequency of bursts, versus tonic firing, when the animal was actively moving.

The increase in neural responsiveness during locomotion is much greater than generally seen with attentional effects, and may represent an overall activation of cortex during arousal. We have recently reproduced this finding with two-photon calcium imaging in the alert mouse, and by combining this paradigm with labeled populations of defined cell types, we are investigating the circuit mechanisms that generate the response modulation.

Differentiate reproduction and aggression pathways in rodents

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Inter male aggression and male female mating are generally considered as distinct behaviors. Nevertheless, it is increasingly recognized that these opponent behaviors may share similar neurobiological and neuroendocrine mechanisms. For example, both mating and territorial aggression are dependent upon circulating gonadal steroids. Both behaviors rely heavily on olfactory and pheromonal input. Lesions of the medial hypothalamus and medial amygdala in rodents decrease the occurrences of both mating and fighting. Taken together, these data suggest that mating and agonistic behaviors may be subserved by a common network of steroid hormone sensitive limbic areas. It is unclear how these two heavily overlapped pathways produce two opposite behavior outcomes. One possibility is that two intermingled but distinct subpopulations of neurons mediate mating and fighting. Alternatively, the same population of neurons mediates both mating and fighting through neuromodulation. The goal of this current study is to distinguish these two signal processing scenarios. We first performed between animal comparisons of the patterns of brain activation during mating and fighting, using c-fos analytic methods that permits rapid sampling across the entire brain. Our results indicate that mating and fighting indeed activate many similar hypothalamic and amygdalar regions in mice. However these areas are distinct from those activated during anti-predator defense. Next, we adapted a method to compare c-fos expression induced during the two behaviors in the same animal. Our data suggest that, at least in some commonly activated regions, two largely distinctive sets of neurons are likely involved in mating and fighting, while the same behavior tends to recruit a stereotyped set of neurons. Given our poor understanding of the aggression circuit in general, we decided to further investigate the functional roles of several hypothalamic regions in aggression based on our Fos results. Using reversible viral inactivation tools, we found that neurons in the ventrolateral region of the ventromedial hypothalamus and its surrounding regions are likely to be critical for aggression initiation. Finally, we used chronic recording in awake behaving animals to understand the physiological responses of those neurons during various episodes of aggressive and sexual behaviors.

THE HABENULO-INTERPEDUNCULAR PATHWAY AS A POSSIBLE SWITCH FOR THE CHOICE OF FEAR RESPONSES IN ZEBRAFISH.

Hitoshi Okamoto, Masakazu Agetsuma, Hidenori Aizawa, Tazu Aoki, Ryunosuke Amo, Ryo Aoki

RIKEN Brain Science Inst., Lab. for Developmental Gene Regulation, 2-1 Hirosawa, Wako, Saitama, 351-0198, Japan

Recent progress in understanding of vertebrate brain development has suggested that the neural circuits regulating fear behaviors may be highly conserved among all vertebrates. The habenula is a part of such evolutionarily highly conserved neural circuits that connects telencephalic nuclei to the interpeduncular nucleus (IPN) of the midbrain. We have recently elucidated that zebrafish has the equivalent substructures as the mammalian habenula, i.e. the dorsal habenula of zebrafish corresponds to the medial habenula of mammals, while the ventral habenula of zebrafish corresponds to the lateral habenula of the mammals (J. Neuroscience. in press). In addition, we already showed in zebrafish a prominent asymmetric habenulo-interpeduncular projection caused by a prominent left-right (LR) difference in the size ratio of the medial and lateral subnuclei of the dorsal habenulae, each of which specifically projects either to the ventral or dorsal IPN targets (Aizawa et al., Current Biol. 2005, Dev. Cell. 2007). Furthermore, we have recently discovered that the neurons in the dorsal IPN specifically send the descending axons along the periaqueductal gray (PAG) in the hindbrain, and the neurons in the ventral IPN project to the raphe. Considering that the PAG is involved in choice of innately encoded defense behaviors such as freezing, flight and fight and that the raphe and its serotonin neurons are involved in regulation of adaptive behaviors, the dorsal and ventral IPN may be involved in the alternative behavioral choice in the face of danger among innate and adaptive programs.

To further investigate the physiological meaning of this prominent asymmetric axonal projection pattern, we have established the transgenic zebrafish line in which the neural signal transmission by way of the lateral subnucleus of the dorsal habenula is selectively impaired either constitutively or conditionally. In the fear conditioning, the manipulated fish showed extremely enhanced levels of freezing response to presentation of the conditioned stimulus, very similarly as human patients suffering from PTSD (post-traumatic stress disorder). This result suggests the tract connecting the left-dominant lateral subnuclei of the dorsal habenula with the dorsal IPN may normally function to experience-dependently suppress the choice of excessive freezing as a response toward conditioned fearful stimuli.

Dense reconstruction of bipolar and ganglion cells in the IPL of mouse retina using serial blockface scanning electron microscopy (SBFSEM) and semi-automated neurite reconstruction

Moritz Helmstaedter[1], Kevin L Briggman[1], Viren Jain[2], Srinivas Turaga[2], Sebastian Seung[2], Winfried Denk[1]

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The mapping of neuronal connectivity is one of the main challenges in systems neuroscience. Now that the acquisition of large 3D EM volumes has been automated by SBFSEM, the most time consuming aspect of circuit mapping is the tracing of neuronal processes and the identification of putative synapses. At the moment, these analysis steps far exceed the time it takes to actually acquire the data volumes. We have therefore developed methods to speed up the manual tracing of processes and semi-automate the detection of contacts between cells. Efficient detection of such physical contacts between neuronal processes is a prerequisite for the automated identification of putative synaptic contacts. Here, we analyze neurite-to-neurite contacts at the EM level in the inner plexiform layer (IPL) of the mouse retina. We have imaged a block of 120x120x80µm, spanning the ganglion cell layer to the photoreceptor layer, at 16.5x16.5x25nm resolution using SBFSEM. We have reconstructed approximately 200 bipolar cells and dozens of ganglion cells in that volume by the consolidation of manual neurite skeletons from multiple human tracers. The neurite skeletons were then grown into volume reconstructions using the automated classification output of convolutional neural networks. An automated method next analyzes the features of the contact areas between touching neurites. We are currently analyzing the pattern of these contacts between bipolar and ganglion cell neurites. This data provides information on both the prevalence of neuron types, on their stratification patterns in the IPL, and eventually on the probability of contacts between costratifying bipolar and ganglion cells at EM resolution.

(MH and KLB contributed equally)

WHAT CAN CONNECTOMICS TELL US ABOUT CORTICAL RECEPTIVE FIELDS?

Davi Bock[1,2], Wei-Chung A Lee[1,2], Aaron Kerlin[1], Mark L Andermann[1], Greg Hood[3], Art W Wetzel[3], Ed Soucy[2], Sergey Yurgenson[1], R. Clay Reid[1,2]

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The rich history of visual physiology depends greatly on the phenomenon of receptive fields, but despite a half century of work, how receptive fields are constructed from the visual cortical circuit remains unclear. A key reason for our lack of understanding is that we do not have the benefit of a single wiring diagram from any cortical circuit. To investigate the interplay between circuit structure and neuronal function we combined single-cell resolution functional imaging with new techniques for high-throughput, large-scale electron microscopy. We recorded the in vivo response properties of neurons in mouse primary visual cortex using two-photon calcium imaging, then prepared the brain for ultrathin (< 50 nm) sectioning. We found the same neurons as targets for reconstruction by serial section Transmission Electron Microscopy (ssTEM). Using a custom-built, high-throughput TEM Camera Array (TEMCA), we acquired tens of terabytes of EM image data, spanning five million cubic microns of brain capturing the superficial layers of visual cortex at vesicular resolution. The EM dataset was globally registered using newly developed automated methods for aligning large stacks of TEM images and traverses ~ 1500 cell bodies for which we know the orientation tuning of a subset. Now we can ask what, if any, rules of anatomical connectivity govern neuronal function.

Acknowledgements:
This work was supported by the Connectome Project at the Center for Brain Science at Harvard, and by Microsoft Research.

A 3D Compartment-Level Neuronal Wiring Atlas of the Adult Fruit Fly Brain and Its Informatics Pipeline

Hanchuan Peng

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We built a compartment-level 3D digital neuronal atlas of an adult fruit fly brain. In this model we quantitatively describe the distribution of neurite structures of an entire set of 64 brain compartments labeled in a collection of 500 GAL4 lines (H. Simpson lab), as well as an initial brain wiring diagram how neurites connect and project throughout these compartments. We computationally analyzed this atlas using neurite patterns derived from another set of hundreds of GAL4 lines (G. Rubin lab). To produce this informative atlas, we developed a pipeline of image analysis and informatics tools. Given a set of 3D confocal image stacks of fruit fly brains of many neuronal GAL4 lines, our pipeline first registered these images accurately in 3D using a non-rigid warping. As a result, we can now compare fly brains in the same coordinate system. We thus identified a subset of 100 GAL4 lines that have relatively sparse neurite patterns. Then, we developed an efficient 3D neuron reconstruction method to digitize the morphology and location of neurite structures in each selected GAL4 lines. These neurites as a whole indicate the projection and putative connection of neurites among brain compartments. We also evaluated the stereotypy of neurites contained in this atlas. Our data indicate that the variation of neurite throughout the central complex of a fly brain has a range about 0.5 to 3 um. Our informatics framework can also be applied to studying neuronal circuits of other animals.
(This work is based on a collaboration with Julie Simpson, Gerry Rubin, and Gene Myers labs)

THE SYNAPTIC CIRCUITS OF THE FLY'S MEDULLA COLUMN

Ian A Meinertzhagen[2], Shinya Takemura[1], Zhiyuan Lu[2], Richard D Fetter[1], Shiv Vitaladevuni[1], Louis K Scheffer[1], Dmitri B Chklovskii[1]

[1]HHMI, Janelia Farm Research Campus, 19700 Helix Drive, Ashburn, VA, 20147, [2]Dalhousie University, Psychology and Neuroscience, Life Sciences Centre, Halifax, NS, B3H 4J1, Canada

Synaptic circuits in the *Drosophila* brain are now beginning to yield to new methods of dense reconstruction using serial-EM approaches in favorable neuropils. The medulla of each optic lobe occupies $10^6 \mu m^3$, about 14% the volume of the entire brain's neuropils, and has > 60 morphological cell types. Against these deterrent features should be set the fact that: a) the medulla is arranged in ~750 columns with a repeating structure of input elements from the first neuropil, the lamina; b) the anatomical circuits of the lamina are already identified; c) the medulla is layered in 10 strata that are respected by both the terminals of lamina input neurons and the arbors of medulla cells; and d) the latter cells are morphologically highly determinate. We identify medulla cells with reference to previous reports from Golgi impregnation, or more recent data from genetic reporters using the Gal4/UAS system. From a series of >1950 40-nm EM sections, a 100 x 100µm area has been imaged and aligned from ~1800 sections, from which we have reconstructed an area 9 x 9µm covering an entire column for 50µm in the z-axis, through strata M1-M10. In this volume we have reconstructed >32 columnar cells, having axons that run down the column axis. These include 10 input terminals from: 5 lamina cells, L1-L5, 2 photoreceptors, R7 and R8, and 3 other neurons (medulla cell T1, and centrifugal cells C2 and C3). From their reconstructions and ultrastructural criteria, we have documented synaptic pathways within the column. Synapses are mostly triads and localize to input terminals. The shapes of the medulla neurons conform closely to previous Golgi reports and GFP labeled profiles, providing assurance that our reconstructions are largely complete and accurate. We have identified medulla neuron targets for various lamina pathways. For the color pathways from R7 and R8, three are: amacrine neuron Dm8, which receives pooled R7 input; Tm5; and Tm9 (Gao *et al*., Neuron 2008). L1 and L2 play crucial roles in motion detection. Their major targets are: for L2, Tm1 and Tm2, frequent partners at postsynaptic triads; and for L1, Mi1 and other neurons (Tm3, C3, L5), one of which (L5) connects reciprocally to L1. L4 is postsynaptic to Tm2, sharing this target with L2. L1 and L2 have segregated medulla pathways connected only via the two centrifugal cells, C2 and C3; with both cells also providing input to L5. Most synapses in this dense neuropil lack functional explanation, and can only be resolved by EM. Identifying their circuits requires serial EM, a lengthy but accelerating process, for which we report the first analysis of a single medulla column.

Building Receptive Fields for Optic Flow Based Navigation:
A Simulation Study of the Fly Lobula Plate Network

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Optic flow based navigation is a fundamental way of visual course control described in many different species including man. In the fly, an essential part of optic flow analysis is performed in the lobula plate, a retinotopic map of motion in the environment with the four cardinal directions (down, up, rightward, leftward) represented in four different layers. There, a small a small network of identified neurons, the so-called lobula plate tangential cells, extend their dendrites in different parts of the map and in different layers to receive motion input. The lobula plate tangential cells possess large receptive fields with different preferred directions in different parts of the receptive field, the structure of which resembles an optic flow as elicited by certain flight maneuvers. However, their dendritic fields within the lobula plate is much smaller and confined, in most cases, to only one layer, suggesting a rather restricted receptive field with a uniform preferred direction. On the other hand, a recent set of studies in blowflies and in Drosophila demonstrated an extensive connectivity between different tangential cells, providing, in principle, the structural basis for their complex receptive fields. Here, I present a network simulation of the lobula plate tangential cells, comprising all the neurons studied so far (22 on each hemisphere) with all the known connectivity based on gap junctions as well as excitatory and inhibitory chemical synapses. The dendrite of each these model neurons receives input from a retinotopic array of Reichardt-type motion detectors according to the location of the dendrite within the lobula plate. When probing the receptive fields of these model neurons, they exhibit flow fields much like their natural counterparts, demonstrating that the connectivity between the lobula plate tangential cells indeed can quantitatively account for their complex receptive field structure. When tested with global motion patterns corresponding to different types of ego-motion (e.g. yaw, roll, pitch), each of these model neurons can be shown to respond optimally to a particular flight maneuver.

THE HABENULA REGULATES EXPERIENCE-DEPENDENT CHOICE OF FEAR RESPONSES IN ZEBRAFISH.

Masakazu Agetsuma[1], Hidenori Aizawa[1], Tazu Aoki[1], Mikako Takahoko[1], Ryoko Nakayama[1], Takayuki Sassa[1], Toshiyuki Shiraki[1], Midori Goto[1], Koichi Kawakami[2], Shin-ichi Higashijima[3], Hitoshi Okamoto[1]

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Under threatening environments, choice of a suitable response is critical for survival. Animals exposed to natural predators elicit a freezing or flight response, with the choice between them depending on multiple factors including species, environment, age, and experiences (Bandler et al., 2000; Eilam, 2005; Fanselow, 1994). For example, different parts of septo-hippocampal areas are activated after the different experience, by which animals elicit a freezing or flight response, and a similar experience-dependent predominance of a specific region was also seen in the cortical, amygdalar, striatal, and hypothalamic areas (Mobbs et al., 2007; Mongeau et al., 2003). It is also shown that different parts of periaqueductal gray (PAG) differentially regulate coping strategies against stress (Bandler et al., 2000; Fanselow, 1994). Both the rostral and caudal parts of the lateral or dorso-lateral PAG are responsible for active coping of stress, with the rostral parts evoking confrontational defensive posture and the caudal parts evoking flight escape behaviors. In contrast, the ventrolateral PAG is responsible for a passive coping reaction with freezing. However, in spite of the progress in the study of respective networks, the mechanisms underlying the decision of which circuit to elicit for the behavioral choice remain unclear.

Here we show that such choice critically depends on the habenula, an evolutionarily highly conserved diencephalic structure. In zebrafish, the lateral subnuclei of the dorsal habenula (dHbL) are asymmetrically connected with the dorsal and intermediate parts of the interpeduncular nucleus (d/iIPN). We demonstrated this pathway further projects to the periaqueductal gray (PAG), and the specific silencing of this pathway rendered animals extraordinarily prone to freeze against conditioned fear stimuli, while the control fish showed only flight behaviors. There was no difference in the basic locomotor activity and the US sensitivity during the fear conditioning tasks. The modification of fear behaviors by this pathway is experience-dependent and is versatile for various sensory inputs. These results demonstrate a novel and crucial role of the dHbL-d/iIPN-PAG pathway in the choice of fear responses.

DISRUPTION OF PATTERNED SPINAL ACTIVITY FOLLOWING ACUTE CONTUSIVE SPINAL CORD INJURY

Zaghloul Ahmed

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Patterned activity by spinal motoneurons are generated in response to inputs from local circuits of excitatory and inhibitory interneurons. Spinal cord injury has been shown to affect the activity of these circuits by disturbing the local connections and by depriving them of supraspinal modulatory inputs. The aim of this study is to assess, in vivo, the effect of acute contusive L1 spinal cord injury on the excitability and rhythmic bursting of spinal motor neurons generated by local spinal injection (L3-L4) of inhibitory neurotransmitters blockers (GABA and glycine). These blockers generated strong synchronized bursts that were recorded from the tibial nerve before, through, and following spinal cord injury (using NYU impactor). Spinal cord injury completely abolished all neuronal activity including the synchronized bursts for about 12±3.8 minutes following the injury (n=7). Bursting gradually appeared, but with a qualitative and dramatic change. Compared to before injury, these recovered bursts showed reductions in frequency, number of sub bursting, spike amplitude, and burst duration. The strength of bursting, measured as the peak at 10ms in the autocorrelation histogram, was significantly weaker after injury. In addition, the strength of burst oscillation, defined as the difference between the second trough-to-peak oscillation, was significantly weaker. After injury, the autocorrelation analysis also showed significant increase in the strength of the bursting refractory period. In this preparation, before injury, we were able to induce bursting by electrical stimulation of the sciatic nerve, however, after injury electrical stimulation could not induce bursting despite increasing stimulus strength, indicating decreased excitability of spinal networks. All changes persisted for two hours, the time of the experiment. In additional experiments (n=2), injecting the blockers following acute injury induced weak and not oscillating bursts. This indicates that the changes mentioned above were not resulted from an interaction between the blockers and the injury. These results quantify, in vivo, important changes in the spinal networks and also provide a model to study spinal shock and recovery after contusive spinal cord injury.

Modality specific homeostatic synaptic plasticity in the developing optic tectum

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Synaptic scaling is a type of bidirectional, homeostatic synaptic plasticity in which the strengths of all the synapses in a neuron are adjusted in response to global changes in neural activity. However, it remains unclear whether a similar type of scaling could occur in an input-specific manner. Here we address this issue by studying synaptic scaling of inputs conveying different sensory modalities and converging onto individual neurons in the optic tectum of Xenopus laevis tadpoles. The optic tectum, a structure homologous to the superior colliculus, integrates information from various sensory modalities. In Xenopus, the tectum receives visual input from the contralateral retina. It also receives ascending projections from primary sensory nuclei in the hindbrain which convey information from several other mechanosensory modalities. During development, these inputs innervate the tectum at the same time as visual inputs begin to mature. Using an isolated whole-brain preparation we can electrically isolate visual and mechanosensory inputs, while performing whole-cell recordings from tectal neurons. We find that most tectal neurons in stage 42 - 49 tadpoles receive direct input from both pathways. To induce synaptic scaling, we placed tadpoles in the dark for 48 hrs. We then tested visual and mechanosensory inputs separately by stimulating the pathways under conditions in which extracellular Ca++ had been replaced by Sr++ to induce asynchronous neurotransmitter release. This allowed us to resolve quantal events (referred to as asynchronous EPSCs or asEPSC) from each of the stimulated pathways independently. 48 hours in the dark resulted in a selective enhancement of asEPSC amplitudes from visual inputs but not mechanosensory inputs. While a decrease in activity is known to result in scaling up of synaptic weights, an overall increase in activity causes a scaling down of synaptic weights. To test whether scaling down could be input specific, we reared tadpoles for 48 hours in an normal light/dark environment but continuously bubbled air into the rearing media to induce an elevated level of vibratory stimuli to activate the mechanosensory pathways. This resulted in decreased asEPSC amplitudes evoked form the mechanosensory but not visual pathways. Global scaling could be induced in all inputs if inhibition was pharmacologically blocked for 48 hrs. Taken together these experiments suggest that homeostatic synaptic plasticity can function at the level of individual inputs to a neuron depending on their overall long-term activity levels. Furthermore, these experiments also show that the tadpole tectum can be a useful preparation to study the development and plasticity of multisensory neurons.

REPLAY OF IMAGE SEQUENCES IN ALERT MONKEY V4

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It has been shown that individual neurons in many brain regions display the same spiking pattern of previously evoked activity in the absence of further external stimulation – this is known as replay or reverberation. Neuronal replay has been proposed to constitute a mechanism of memory consolidation among neural ensembles which could characterize neuronal responses in many cortical areas. However, whereas replay of neuronal activity has been mainly described in the hippocampus and prefrontal cortex, whether this phenomenon can be found in visual cortex is unclear. Our objective was to induce the replay of neuronal activity in macaque area V4 by exposing multiple neurons to portions of natural images briefly flashed for 120 ms each in a random spatio-temporal sequence (the total stimulus duration was 3s). Each stimulus trial was followed by a blank trial of similar duration (each session had approximately 150 blank and stimulus trials). Additionally, we presented 30 blank trials at the beginning and end of each session to assess differences in activity with stimulation. Since the individual images stimulated the receptive fields of the neurons in a specific temporal pattern, the stimulus caused neurons to fire in a temporal sequence. Analysis of the replay was performed by examining the neuronal responses across the population of cells in the blank trials that followed each stimulus presentation. We examined whether the temporal population response seen in the blank trials resembled the population response during the stimulus trials. We assessed the degree of similarity between the neuronal responses in the blank and stimulus trials by computing the correlation coefficient between the two sets of response patterns. Bootstrap analysis revealed significant correlations in 83% of the sessions. Furthermore, correlations between the prestimulus blank period and stimulation period were less than that between the stimulus and blank periods, indicating a significant change in the population response pattern with stimulation. Importantly, we found that neuronal replay in visual cortex is stimulus specific and that the effect is greatly reduced when the population size was decreased. Altogether, these findings demonstrate successful induction of reverberation in primate V4 in the absence of visual stimulation.

Identification of the zebrafish ventral habenula as a homologue of the mammalian lateral habenula

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Mammalian habenula consists of the medial and lateral habenulae. Recent behavioral and electrophysiological studies suggested that the lateral habenula plays a pivotal role in controlling motor and cognitive behaviors by influencing the activity of dopaminergic and serotonergic neurons. Despite the functional significance, manipulating neural activity in this pathway remains difficult, due to the absence of a genetically accessible animal model such as zebrafish. To address the level of lateral habenula conservation in zebrafish, we applied the tract tracing technique to GFP-expressing transgenic zebrafish to identify habenular neurons that project to the raphe nuclei, a major target of the mammalian lateral habenula. Axonal tracing in live and fixed fish showed projection of zebrafish ventral habenula axons to the ventral part of the median raphe, but not to the interpeduncular nucleus where the dorsal habenula projected. The ventral habenula expressed *protocadherin 10a*, a specific marker of the rat lateral habenula, while the dorsal habenula showed no such expression. Gene expression analyses revealed that the ventromedially positioned ventral habenula in the adult originated from the region of primordium lateral to the dorsal habenula during development. This suggested that zebrafish habenulae emerge during development with mediolateral orientation similar to that of the mammalian medial and lateral habenulae. These findings indicated that the lateral habenular pathways are evolutionarily conserved pathways and might control adaptive behaviors in vertebrates through the regulation of monoaminergic activities.

Optogenetic probing of the oculomotor system in zebrafish

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Expression of halorhodopsin (NpHR), a light-driven microbial chloride pump, allows for optical control of membrane potential and reversible silencing of transfected neurons. Zebrafish are ideal model organisms for optogenetics, since they are optically clear, genetically tractable and display many behaviors within the first two weeks of life. We study the neural circuits underlying the optokinetic response (OKR), a visual behavior, which minimizes retinal slip during rotating motion.
Applying light through thin optic fibers positioned above the animal's head enabled us to target small groups of cells and to simultaneously test the effect of their silencing on the generation of saccades during the OKR. We identified a cell population in close proximity to the cranial nucleus VI that is required and sufficient for the generation of fast eye movements (saccades) in zebrafish larvae. Bilateral stimulation of NpHR suppressed saccades completely but left slow phase eye movements unaffected. Unilateral stimulation blocked saccades of both eyes in the direction of the stimulated side, but not in the other direction. Conversely, ChR2-mediated activation of this neuronal population elicited full-blown saccades in both eyes. In an unrelated forward-genetic screen (Muto et al., PLoS Genet. 2005), our group had identified a voltage-gated sodium channel mutant, *didy*, with a specific deficit in sustaining saccades. The saccade deficit in mutants could be rescued by locally activating ChR2, which suggested that the deficit is located upstream of muscles and motoneurons.
Furthermore, we studied the cardiac conduction system throughout development using these tools. By automated sequential illumination of small areas of the developing heart with a digital micromirror device (DMD), while simultaneously monitoring heart contractions, we identified the regions responsible for initiating and relaying cardiac conduction. By directly targeting the pacemaker areas with well-defined light pulses, the heart rate can be increased with ChR2 stimulation and decreased to a full stop with NpHR stimulation.
Together, our studies introduce an optogenetic toolkit for precise loss-of-function and gain-of-function analysis of neural circuits and behavior.

OPTOGENETIC ANALYSIS OF NEOCORTICAL MICROCIRCUITS IN LAYER 2/3 OF MOUSE PRIMARY SOMATOSENSORY BARREL CORTEX *in vitro*.

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Neocortical microcircuits are thought to contribute importantly to the sophisticated processing abilities of the mammalian brain. Neurons within the neocortex communicate with each other through releasing the excitatory neurotransmitter glutamate or the inhibitory neurotransmitter GABA. In order to begin to understand the functional operation of the neocortex, it will clearly be important to establish the basic organizing principles of synaptic connectivity between different classes of cortical neurons. Here we express the light-gated cation channel channelrhodopsin-2 (ChR2) (Nagel et al. 2003; Boyden et al. 2005) in excitatory layer 2/3 neurons of primary somatosensory barrel cortex to investigate circuit function underlying whisker perception.
To express ChR2 in barrel cortex pyramidal neurons, we used a lentivector encoding a ChR2-mCherry fusion protein under the control of the CaMKII promoter (Zhang et al. 2007). The lentivector was injected into layer 2/3 barrel cortex of GAD67-GFP knock-in mice, which express GFP in GABAergic interneurons (Tamamaki et al. 2003). After 4-6 days to allow expression of ChR2, *in vitro* brain slices were prepared. ChR2 expressed in layer 2/3 pyramidal cells was confined to within ~200μm of the injection site. We made whole-cell recordings from neurons expressing ChR2 and from nearby non-expressing neurons targeted by two photon microscopy.
ChR2-expressing neurons responded with rapid depolarization, when illuminated with blue light and action potentials could be triggered reliably with millisecond precision. In nearby non-expressing neurons, blue light also evoked a depolarizing response, but with longer latencies and the response could be blocked by application of the ionotropic glutamate receptor antagonists CNQX and APV. The long-latency response in non-expressing neurons was therefore driven by glutamatergic synaptic transmission evoked by the action potentials in the ChR2-expressing neurons. The amplitude and kinetics of the postsynaptic potentials (PSPs) varied depending upon the type of postsynaptic cell. Fast-Spiking (FS) GABAergic neurons responded with significantly larger amplitude depolarizing PSPs compared to both excitatory pyramidal neurons and Non-Fast-Spiking (NFS) GABAergic neurons. Fast-Spiking (FS) GABAergic neurons were found to be parvalbumin positive, whereas Non-Fast-Spiking (NFS) GABAergic neurons did not express parvalbumin.
In many cases, the ChR2-mediated excitation of presynaptic pyramidal neurons evoked a sequence of excitation-inhibition in their postsynaptic targets. This disynaptic inhibition is likely to be mediated by recruitment of FS GABAergic neurons. Not only did FS GABAergic neurons receive larger amplitude depolarizing PSPs, but they were also more likely to fire synaptically-driven action potentials. In agreement with previous studies, our results suggest that FS GABAergic neurons in layer 2/3 mouse barrel cortex appear well-suited to mediate rapid feedback inhibition.

Building a functional map of the *Drosophila* color vision system

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The interactions between the cell types that comprise a sensory neuronal circuit enable an organism to perceive information from its surroundings and produce an appropriate behavior. Defining the contribution of each of these cell types to the logic of the circuitry is essential to our understanding of how sensory information is processed. The *Drosophila* visual system, with its relatively simple anatomy and its amenability to genetic manipulation is ideal for such studies. The aim of this work is to decipher how the neurons of the fly medulla, the main color vision processing center in fly brain, process color information coming from the sensory end of the circuit, the photoreceptors. We have developed an *in vivo* system in which the activity of medulla neurons in response to a specific wavelength of light is measured using whole-cell patch-clamp recording techniques. We are combining our detailed knowledge of the anatomy of the optic lobe and the availability of specific marker lines to target our recordings to specific neuronal types using GFP expression. In preliminary experiments we have been able to record spontaneous as well as evoked activity of medulla neurons. We are now focusing our efforts on the *ort* positive cells of the medulla, which are post synaptic to the photoreceptors. This work will enable us to build a functional map of the color vision system and will lead to an understanding of how color is represented in the brain.

Hardware for Optical Perturbation of 3-D Neural Circuits: Towards High-Throughput Screening of Neural Circuit Targets

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A key feature of neural circuits in the mammalian brain is their 3-dimensionality and geometric complexity. Hardware that enables the optical silencing or driving of neural activity in complexly-shaped brain circuits would enable analysis of the time-resolved contribution of specific neural circuits and activity patterns, to normal and pathological behavior. We here present a suite of hardware technologies capable of multiscale optical neural control, aiming towards abilities such as bilateral inactivation of the entire CA1 field of the mouse hippocampus or lamina-specific addressability of cortical neurons. First, we developed arrays of LED-coupled 200 μm optical fibers, which are end-user customizable, that allow delivery of light to tens of sites in the brain. Very long duration operation is possible via modular fluidic cooling, e.g. 10 LEDs for 30 continuous seconds or 3 LEDs indefinitely. The arrays and cooling systems together weigh <2.5 grams, light enough to be borne on the head of a freely moving mouse. We have also developed electrodes and circuits that allow concurrent neural recording impervious to noise from the LEDs. Second, we present the design and implementation of mass-fabricatable multi-lightguide microstructures, produced using standard microfabrication techniques. Each microstructure is a 200-micron wide insertable probe comprising many 20 μm wide lightguides running in parallel, and capable of delivering light to many points along the axis of insertion of the probe, akin to existing silicon probes.

We demonstrate implantation of high-count fiber arrays to complex 3-D circuits in the mammalian brain. These technologies will, for example, enable high-throughput screening of neural circuits important for behaviors, thus identifying neural targets from a functional point of view. As a first step towards such a systematic screen, finding neural circuits and activity patterns that can alleviate specific forms of anxiety and post-traumatic stress disorder in a mouse model, we show that we can facilitate the extinction of Pavlovian fear conditioning through manipulation of selective cortical targets in freely moving mice, evidenced by freezing decrease of 73% compared to control mice at the end of the first session of extinction learning ($p<.02$, $n=5$).

Do gap junctions play a role in propagation of retinal waves?

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In the days before eye opening in mice, retinal waves, the spontaneous activity found in the developing retina, propagate laterally among bipolar cells, which are not synaptically connected. However, bipolar cells do form electrical synapses with interneurons using two connexins, Connexin 36 (Cx36) and Cx45 . To test whether retinal waves propagate among bipolar cells via gap junctions we have studied waves in transgenic mice lacking these connexins. In previous studies our lab showed that although Cx36ko mice had higher uncorrelated spontaneous firing, they exhibited propagating retinal waves. Here we studied retinal waves in a Cx45ko mouse in which Cre-recombinase expression, driven by the neuron-directed Nestin promoter, deletes Cx45 and activates a GFP reporter. We found that during the first two postnatal weeks, GFP was broadly expressed in the inner nuclear layer and sparsely expressed in the retinal ganglion cell layer in a pattern similar to that of the adult. Using calcium imaging and whole cell recordings, we found that Cx45ko retinas exhibited normal spontaneous activity patterns. To test whether compensation occurs in single-connexin knockout mice, we generated a Cx36-Cx45 double knockout (Cx36-45dko). Whole cell recordings and calcium imaging revealed that Cx36-45dko retinas also exhibited normal retinal waves. Hence, although Cx36 and Cx45 are expressed during development, they do not play a role in the propagation of spontaneous activity.

CORRELATING FUNCTION AND STRUCTURE USING TWO-PHOTON LASER SCANNING AND SERIAL BLOCK-FACE SCANNING ELECTRON MICROSCOPY

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A comprehensive understanding of the function of neuronal circuits requires knowledge of both the response properties of individual neurons and the connectivity amongst them. We have developed a method to bulk load mammalian retina with synthetic fluorescent calcium indicators to record from populations of retinal ganglion cells (RGCs) in response to patterned light stimuli with two-photon laser scanning microscopy. This technique allows us to rapidly characterize the receptive field properties of RGCs and search for cells that respond to specific stimuli, such as directionally-selective (DS) RGCs. The technique does not require penetration with an electrode into the retina and so the ultrastructural integrity of the tissue is not affected.

Following the functional characterization of the receptive fields of approximately 600 RGCs from a piece of mouse retina, we preserved the piece and processed it for serial block-face scanning electron microscopy (SBFSEM). We used a staining protocol designed to render cell surfaces electron dense using horseradish peroxidase and diaminobenzidine. A SBFSEM volume large enough to contain the complete dendritic trees of the recorded RGCs (320µm x 320µm x 60µm) was acquired spanning the ganglion cell layer to the inner nuclear layer at a xy resolution of ~16nm and section thickness of 25nm. We are currently analyzing the pattern of contacts between functionally characterized DS RGCs and presynaptic starburst amacrine and cone bipolar cells.

CHARACTERIZING THE MORPHOLOGY OF CORTICOGENICULATE NEURONS IN THE MONKEY VISUAL SYSTEM

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The feedback circuits connecting sensory cortical areas with the thalamus are ubiquitous in mammalian systems, however the function and organization of such pathways remain poorly understood. We recently demonstrated that the corticogeniculate pathway of the macaque monkey, connecting layer 6 in primary visual cortex with the lateral geniculate nucleus (LGN), is comprised of three functionally distinct cell types with physiological relationships to the feedforward magnocellular, parvocellular, and koniocellular streams (Briggs and Usrey, 2009). In the current study, we provide anatomical evidence in support of a multi-partite organization of this corticogeniculate pathway. We utilized two experimental strategies to characterize the dendritic morphology of corticogeniculate neurons. First, G-deleted Rabies virus containing a GFP construct was injected into the LGN and, acting as a monosynaptic retrograde tracer, generated GFP expression in labeled corticogeniculate neurons. Second, fluorescent dextrans of different colors were injected into the magnocellular and parvocellular layers of the LGN and retrograde-labeled corticogeniculate neurons were photo-stained in living brain slices. Both methods provided robust labeling of corticogeniculate neurons such that the dendritic structure of individual neurons could be reconstructed at a fine level of detail. Our results demonstrate that the morphological characteristics and sub-laminar organization of most corticogeniculate neurons are consistent with their participation in distinct cortical circuits (e.g. magnocellular and parvocellular pathways). In addition, we observed a number of unusual morphological types and novel organizational strategies, suggesting that the corticogeniculate projection may include a larger diversity of cortical circuits than previously thought.

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DOPAMINERGIC MODULATION OF LOCOMOTORY BEHAVIOUR

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Caenorhabditis elegans locomotion consists of a smooth sinusoidal movement on an agar surface. This pattern results from alternating ventral and dorsal contractions of the longitudinal body wall muscles, which are organised into 2 dorsal and 2 ventral rows.

Many mutants with abnormal patterns of locomotion have been identified. Among these are mutations affecting the dopaminergic neurotransmitter system. At present the body mechanics and neural activity underlying normal and defective worm locomotion are not well-characterised. We are investigating how the neural circuitry underlying locomotion controls locomotory behaviour and how this circuitry is modulated by dopamine.

We are using the Worm Tracker 2.0 system developed in the Schafer lab to quantitatively describe the locomotion of wild-type worms at various developmental ages and worms in which dopamine signalling is defective. This is a feature-rich single worm tracker that allows the rapid and consistent quantification of the effects of mutations on behaviour. We are planning to combine this analysis with GCaMP3 calcium imaging data from motor neurons and body wall muscle in freely-moving worms. This makes it possible to investigate the relationship between motor neuron activity, muscle activity and worm shape during locomotion.

The intrinsic properties of the AII amacrine interneuron may differentially shape transmission of rod signals to ON and OFF cone bipolar cells in the mammalian retina

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In the rod pathway of the mammalian retina, a critical interneuron called the AII amacrine cell divides rod-derived signals into parallel ON and OFF cone pathways. Through AIIs, input from rod bipolar (RB) cells is transmitted to ON cone bipolar (CB) cells through electrical synapses and to OFF CBs through glycinergic chemical synapses. At both synaptic and single-cell levels, properties of this circuit exhibit nontraditional behavior. Synaptically, exocytosis from RBs and AIIs occurs at analog synapses whereby small changes in membrane potential may be converted to changes in persistent neurotransmitter release. Intrinsically, AIIs have an elevated resting potential of ~-45mV, which enables the axonless AIIs to fire irregular spikes at rest with somatic amplitudes of ~3-8mV. We sought to examine the mechanisms and functional consequences of these properties from both experimental and theoretical standpoints. To directly assess the contributions of intrinsic AII properties and RB-AII synaptic properties to this microcircuit, we recorded from AIIs and RBs using single and paired whole-cell recordings. We found that the frequency and amplitude of spiking in AIIs, as recorded somatically, was sensitive to the mean membrane potential and enhanced some RB inputs. To complement our experimental findings, we developed a multi-compartmental computational model of the AII that is constrained by experimentally-determined passive and active properties. We employed this model in a network of gap-junction coupled AIIs and delivered simulated RB inputs. To study circuit properties with ON and OFF CBs, we explicitly simulated ON CBs and considered calcium influx at the site of synaptic communication with OFF CBs. We found that after incorporating noise, along with a distal voltage-gated Na conductance, our model may account for the nontraditional AII spiking behavior. Specifically, a single dendritic activation site produces attenuated somatic responses consistent with experimental results. This dendritic conductance may enhance RB signals at the location of input and may differentially enhance synaptic communication with ON CBs and OFF CBs: gap junction transmission to ON CBs located near RB input is elevated, while signals to distal OFF CBs are attenuated and large changes in calcium influx are prevented at this analog chemical synapse.

Genetic dissection of the function of hindbrain commissures: rewiring the brain with Robo3

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In bilateria, many axons cross the midline of the central nervous system forming well-defined commissures. In the visual system and the corpus callosum in the neocortex, the physiological importance of brain commissures is well established in large part due to the “split-brain” studies of Roger Sperry and others. However, the function of commissural projections in the hindbrain and spinal cord has been more difficult to assess . To improve our understanding of the function of hindbrain commissures, we undertook to genetically rewire the hindbrain by forcing select commissural tracts to remain ipsilateral, and then examined the cellular and behavioral consequence of these manipulations. Studies in mice show that in hindbrain and spinal cord, the Robo3 receptor is required for commissural axons to cross the midline and that commissural neurons, deficient in Robo3, extend their axons ipsilaterally. By taking advantage of multiple Cre transgenic lines, we have induced site-specific deletions of the Robo3 receptor. These lines developed with the disruption of specific commissures in the sensory, motor and sensorimotor systems resulting in severe and permanent functional deficits. We show that mice with severely reduced commissures in rhombomeres 5 and 3 have abnormal lateral eye movements and auditory brainstem responses respectively, whereas mice with a primarily uncrossed climbing fiber/Purkinje cell projection are strongly ataxic. Surprisingly, although rerouted axons remain ipsilateral, they still project to their appropriate neuronal targets. Moreover, some Cre;Robo3 lines represent potential models that can be used to study human syndromes, including Horizontal Gaze Palsy with Progressive Scoliosis (HGPPS). To our knowledge, this study is one of the first to link defects in commissural axon guidance with specific cellular and behavioral phenotypes.

STRUCTURE/FUNCTION ANALYSIS OF A NEURAL INTEGRATOR

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The oculomotor integrators are central components in the control of eye position and also serve as model systems for the study of short-term memory. Many proposed models of the mechanisms of integration exist and it has been difficult to determine the most biologically relevant model due to an incomplete picture of the inherent structural constraints of the integrator network. To elucidate the structure/function relationships within the horizontal velocity-to-position integrator, we have developed a novel set of methods that allows for the optical identification of integrator cells and their subsequent targeted dye-loading during behavior.

We have taken advantage of the transparency of the larval zebrafish to perfom two-photon fluorescence imaging of bolus-loaded calcium indicators and inert tracer dyes in addition to performing single-unit electrophysiology, infrared eye tracking, and presentation of optokinetic stimuli all in one preparation.

Using these methods, we have been able to identify integrator cells by measuring strong correlations between their calcium-sensitive somatic fluorescence and eye-position. We verified this identity by loose-patch recording which indicates that changes in fluorescence are roughly linearly correlated with changes in firing rate. We have found a large variations in the position/velocity sensitivities of cells during optokinetic behaviors as well as variations in the decay times during fixations. This heterogeneity in dynamics is not consistent with single-mode models of the integrator. We have also observed elevated calcium signals over broad areas of the neuropil which are associated with saccadic motions.

Finally, we have successfully loaded functionally identified integrator cells with fluorescent dyes of a color different than that of the calcium indicator which has allowed for the visualization of dendritic morphology and axonal projections including spines and boutons. Within minutes after electroporation, eye position-correlated changes in fluorescence of the calcium indicator can still be detected. The capacity to now selectively label identified integrator cells in the awake animal with inert dyes of different colors promises to further our understanding of the structure/function relationship underlying integration.

The absence of synaptically evoked plateau potentials in LGN cells leads to a failure in retinogeniculate refinement.

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Spontaneous retinal waves lead to robust excitatory postsynaptic activity in developing relay cells of the dorsal lateral geniculate nucleus (LGN). Based on our *in vitro* recordings, this activity takes the form of plateau potentials: large, long-lasting, high-amplitude depolarizations mediated by L-type Ca^{2+} channel activation. Plateau potentials prevail early in life and many aspects of developing retinogeniculate circuitry (e.g., the high degree of retinal convergence, the temporal summation of excitatory postsynaptic potentials, and the lack of inhibitory connections) favor their activation. Moreover, the associated Ca^{2+} influx could initiate signaling cascades to activate the transcription of plasticity related genes and the subsequent remodeling of the developing retinogeniculate pathway. The purpose of our present study was to assess whether synaptically evoked plateau potentials in the LGN are necessary for the refinement of retinogeniculate connections. To address this, we used a transgenic mouse that lacks the β_3 subunit of the L-type Ca^{2+} channel. These mutants have far fewer membrane bound L-type Ca^{2+} channels and greatly attenuated L-type activity. In β_3 nulls, L-type plateau potentials are rarely observed in the LGN, even at young ages or when repetitive pulses of electrical stimulation are applied to the optic tract. The few that do occur have different kinetics and seem to rely more on the NMDA receptor than L-type channel activation. We then examined retinal wave activity, the eye-specific patterning of retinogeniculate projections and the degree of retinal convergence in these mutants. *In vitro* multi-electrode array recordings of retina from β_3 null mice reveal normal stage II (cholinergic) and stage III (glutamatergic) spontaneous retinal waves. However, the retinogeniculate projections of β_3 null mice fail to segregate properly. The spatial extent of uncrossed projections is abnormally large and overlaps with crossed ones. *In vitro* thalamic slice recordings also reveal that relay cells in β_3 nulls show less pruning than age-matched wild-types. Relay cells continue to receive as many as 8-10 retinal inputs even as late as P21, a time when wild-type cells typically receive 1-3 inputs. Thus, these results suggest that postsynaptic L-type Ca^{2+} channel activity is necessary to implement the activity dependent refinement of the retinogeniculate pathway.

Towards an Observer Independent Analysis of the Electrical Variability of Cortical Neurons

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The existence of a diversity of electrical classes of cortical neurons is widely agreed upon, yet is essentially based on subjective categorization. Classes such as “regular spiking”, “fast-spiking”, “stutterers”, “bursters” were identified based on the response of neurons to application of depolarizing current steps. Here, analyzing a large database of spiking responses of hundreds of cortical neurons to a standardized set of stimuli, including current pulses, ramp pulses and noisy inputs, we developed methods to objectively assess whether indeed different electrical classes could be identified. These methods include combinations of different dimensionality reduction algorithms (e.g. principal component analysis), statistical analyses and machine learning applications. Together they are used for quantitatively characterizing the firing pattern of a neuron based on “features” of the spiking discharge, such as spike width, time-to-first-spike, spike frequency, degree of “burstiness”, etc. Extracting these features from the database of cells enables us to take into account the variability across different features, different neurons and different putative electrical classes, thus, allowing us to investigate the existence of electrical classes and their coherence in the face of different features. We initially show that parts of the accepted subjective classification, as described by the “Petilla Interneuon Nomenclature Group” (PING), can be recovered using observer independent analysis. However, the classification clearly depends on the features used as the basis of the classification. Thus, we then proceed to consider the utility of different features related to different stimuli. Ultimately, we aim to suggest what would be the “most informative stimulus” set, along with the corresponding features, that provides the best classifier of the electrical classes of neurons and a concise, yet highly informative description of the electrical behavior of the cell.

NEURONAL AND NETWORK MECHANISMS OF ICTOGENESIS

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Epilepsy is the third most common neurological disorder affecting 1% of the American population and 50 million people worldwide. It is a chronic disorder marked by recurrent and often unpredictable seizures. Classic seizure prediction methods use signal processing algorithms on EEG signals to identify the pre-seizure period. However, this approach is inherently non-mechanistic and has produced disappointing results. Our goal is to elucidate the mechanisms that underlie ictogenesis, the process by which normal neural activity synchronizes during the pre-seizure period. We focus on neuronal and network-level mechanisms, as well as how those mechanisms interact to create ictogenic conditions.

At the neuronal level, we hypothesize that the intrinsic properties of individual neurons within a connected population determine a brain region's propensity for ictogenesis. We compare the intrinsic properties of neurons in piriform cortex (PC), a region implicated in temporal lobe epilepsy, to neurons in somatosensory cortex (S1), a region known to be more clinically stable. We also examine how those neurons participate in the onset of an epileptiform event.

At the network level, we hypothesize that the underlying architecture influences and shapes the spatiotemporal patterns of ictogenesis. Using a computational model we show how connection parameters contribute to a network's propensity for ictogenesis. Our simulations yield specific predictions about differences in the network structure of S1 versus PC. Experimentally, we use voltage sensitive dye imaging of cortical slices from S1 and PC to compare their activity patterns during ictogenesis. Our results suggest that the spatially distributed circuitry of PC is reflected in the spatial pattern of activation, whereas S1 activity patterns reflect the recruitment of local circuitry in a smooth, sequential manner.

Finally, we examine the interactions between neuronal and network mechanisms by analyzing an abstract neural population model that incorporates both. Using phase plane and bifurcation analysis, we examine how interactions between membrane and connectivity parameters govern ictogenesis, defined as the behavior of the system near the unstable threshold that separates normal from epileptiform activity.

By focusing on the neuronal and network mechanisms of ictogenesis and their interactions, we are better able to elucidate the sequence of events leading up to an epileptiform event and understand how that sequence relates to the underlying neural substrate. This will allow us to identify specific aspects of the neurophysiology that can be targeted in the development of new clinical therapies. Our intention is to provide a clear and directed strategy for clinical interventions that can stop an epileptic episode before it even starts.

CONTRIBUTION OF SEROTONIN RECEPTOR 1D IN PROPRIOCEPTIVE CIRCUITS TO MOTOR COORDINATION

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Sensory information on muscle length change is detected by proprioceptive sensory neurons innervating muscle spindles. These sensory signals are required for proper locomotion, which is evident in *Egr3*$^{-/-}$ mutant mice that lack muscle spindles and display an ataxic gait (Tourtellotte and Milbrandt, 1998; Nat Genet, 20:87). Apart from sensory innervation, muscle spindles receive efferent innervation from gamma-motor neurons, which regulate the sensitivity of the spindle to length change. Here, we show that serotonin receptor 1d (*5-ht1d*) is selectively expressed in gamma-motor neurons and a subpopulation of proprioceptive sensory neurons. In *5-ht1d*$^{-/-}$ mice, there is no loss of motor neurons and muscle spindles receive both sensory and motor innervation. *5-ht1d*$^{-/-}$ mice show no abnormality in basic locomotor behavior on an accelerating rotarod. However, when provoked to more challenging locomotor behavior, as crossing a narrow round beam and climbing a hanging wire, *5-ht1d*$^{-/-}$ mice show signs of improved coordination abilities. Further electrophysiological, molecular and genetic studies are in progress to determine how this phenotype is related to 5-HT1D signaling in proprioceptive circuits.

Anterograde Jelly belly and neuro-transmitter signalling affect different features of dendritic growth in the motor system of *Drosophila*

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The development of functional neuronal networks requires intricate regulation between exploratory growth of neurites and synaptogenesis through mechanisms of structural growth, retraction, partner recognition, synapse induction, stabilisation and maintenance. We have previously shown that synaptic transmission regulates dendritic growth of motorneuron dendrites during embryonic development in *Drosophila*. Here we demonstrate that Jeb (Jelly belly) is secreted from interneurons presynaptic to motorneurons alongside synaptic transmitter, and that Jeb functions as a second presynaptically derived signal that regulates dendritic growth. Functional inhibition of the Jeb receptor Anaplastic lymphoma kinase (Alk) in the postsynaptic motorneuron alone generates bigger dendritic trees. The opposite manipulation of cell-autonomous upregulation of Jeb/Alk signalling leads to significantly smaller dendritic trees, and this is independent of synaptic transmission. We find that neurotransmission and Jeb/Alk signalling regulate different elements of the dendritic cytoskeleton and thus different aspects of dendritic growth. Jeb/Alk signalling primarily regulates actin-rich, tubulin-void terminal branches of the growing dendritic tree. This may affect the dynamic exploratory growth behaviour of the dendritic arbor and thus its connectivity. Presynaptic excitatory transmitter, Acetylcholine, in contrast, seems to be required for the normal tubulination and thus stabilisation of central sections of the dendritic tree.

We have also previously shown that Protein Kinase A (PKA) signalling is involved in regulating dendritic growth in the embryonic motor system. Others showed that the Alk receptor signals through increased levels of dpERK. The interplay of both pathways in dendritic growth regulation is reminiscent of their role in synaptic plasticity, learning and memory, where they converge on the common downstream integrator CREB. In line with this hypothesis, we find that modulation of CREB activity also affects dendritic growth in embryonic motorneurons. We are currently investigating how these different signalling pathways regulate dendritic growth and synaptic plasticity by working with identified synaptic contacts and will discuss our results.

PRESYNAPTIC $GABA_B$ RECEPTOR SIGNALING REGULATES ACTIVITY-DEPENDENT DEVELOPMENT OF INHIBITORY AXON TERMINALS

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GABA-mediated synaptic inhibition is crucial in neural circuit operations. The development of inhibitory synapses and innervation patterns in the neocortex is a prolonged process, extending well into the postnatal period, and is regulated by neural activity and experience. Such activity-dependent inhibitory synapses development is a major component of the functional maturation of inhibitory circuits, which organize neural ensemble and network dynamics. Activity-dependent GABA signaling has been implicated in sculpting the developmental process of GABAergic interneurons in adolescent brain. However, the underlying cellular mechanisms of this developmental process are still poorly understood. We previously provided evidence for the hypothesis that GABA signaling acts beyond synaptic transmission and regulates inhibitory synapse development. In other words, similar to glutamate signaling at developing excitatory synapses, GABA may coordinate pre- and post-synaptic maturation at inhibitory synapses. Here we address how presynaptic $GABA_B$ receptors ($GABA_BR$) contribute to GABA-mediated regulation of inhibitory synapse development.

By pharmacologically blocking $GABA_BR$ signaling *in vitro* and genetically KO $GABA_BR$ *in vivo*, we found that presynaptic $GABA_BR$ cell-autonomously regulates the axonal and synaptic development in parvalbumin-containing (Pv) basket interneurons. Blocking $GABA_BR$ signaling led to reduction in axon branching and density of presynaptic boutons. To further understand the effect of $GABA_BR$ signaling, we studied the synaptic dynamics with high temporal resolution in Pv basket interneurons using live imaging. We found that small immature presynaptic boutons were unstable. They tended to appear and disappear repeatedly at predefined sites along axons. In GAD67 deficient Pv cells with reduced GABA signaling, there was an increase in the fraction of small unstable boutons. On the other hand, more GABA signaling by over-expressing GAD67 stabilized the unstable small boutons. We also found that $GABA_BR$ agonist rescued the unstable boutons in GAD67 KO cells. Moreover, blocking $GABA_BR$ signaling, either pharmacologically or genetically, decreased bouton stability. These results reveal a novel function of presynaptic $GABA_BR$ signaling in regulating the activity-dependent development of inhibitory innervation by stabilizing immature presynaptic terminals.

Detecting Changes in Spinal Circuitry via Computational Analysis of Locomotor Output

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Current methods of analyzing locomotor output focus on external features, such as cycle period, phase, and rhythmicity. These methods have described the function of the central pattern generator (CPG) circuitry, and have uncovered the respective roles of various interneuron classes. However, in light of anatomical and functional differences at particular levels of the lumbar spinal cord, it is surprising that little is known about the circuitry responsible for these differences. We predicted that a more thorough description of the underlying neuronal circuits could be obtained through a more careful analysis of the output of the circuit—the motor burst, especially when comparing output from different levels of the spinal cord. Therefore, we applied computational methods, including a continuous wavelet transform (CWT) and principle component analysis (PCA), to data from spinal cord ventral root recordings in order to quantify differences in locomotor output between distinct lumbar levels and between wild-type and mutant mice. The CWT allows for a continuous measure of phase, period, and rhythmicity, the metrics commonly used in describing locomotor output. The CWT also allows for the determination of regions of significant strength in the locomotor signal. After using the CWT to align hundreds of motor bursts from a typical recording, we applied PCA to detect principle components and eigenvalues, measures of a characteristic signature reflecting the internal dynamics of motor bursts. The results of PCA change at different levels of the wild-type spinal cord, and therefore reflect and quantify the differences in the underlying circuitry. These analysis techniques have also allowed us to quantify differences in locomotion between wild-type and Lhx3 knockin mutant mice, which are characterized by a respecification of motor neurons in the lateral motor column to a medial motor column subtype (see abstract by Alaynick et al. for discussion of the Lhx3 mutant mouse). These new methods for analyzing locomotor output provide new ways of exploring the circuitry comprising the CPG and will help to standardize phenotypic characterization of mutants.

How worms navigate temperature gradients in extended environments

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The nematode *C. elegans* navigates temperature gradients in search of particular temperatures corresponding to long-term thermotactic memory. In isotropic environments, worm locomotion can be characterized as an alternating sequence of periods of forward movement, reversals, and sharp turns, with stochastic transitions between these different modes of movement. In thermal gradients, the worm uses thermosensory information to modulate its locomotion according to specific rules that constitute thermotactic strategy. Adhering to thermotactic strategy yields movement towards preferred temperatures over time. We have been studying thermotactic strategy in extended two-dimensional environments (20 cm x 20 cm) with defined spatiotemporal thermal gradients, which allows us to collect navigational statistics from individual animals monitored for long periods of time. We use high-resolution videography to track both the time-varying shape and position of individual animals as they respond to temperature gradients while exhibiting different modes of thermotaxis. The extended configuration of our setup now allows us to quantify aspects of navigational strategy with long temporal correlations, as well as gradual changes in thermotactic navigation that unfold over time, providing a far more complete description of the sensorimotor rules that govern exploratory behavior in *C. elegans.*

SINGLE NEURON FEATURES VERSUS NETWORK CONNECTIVITY: A ROBUST NEURAL CIRCUIT MODEL OF THE OCULOMOTOR INTEGRATOR

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The oculomotor neural integrator controls the position of the eyes during fixations and saccades. A prime candidate for horizontal, fixational control are the "position" neurons (PN), which fire persistently with a frequency that is proportional to the horizontal eye position (Major, Tank Curr Opin Neurobiol 2004,14:675–84). Past research has focused on three properties of this system:

(1) Recruitment order: The slope of the PN tuning curves increases as their firing threshold moves towards more eccentric eye positions (Aksay et al J Neurophysiol 2000,84:1035–49).

(2) Hysteresis and fine-tuning: most models of the oculomotor system rely on fine-tuning of synaptic parameters (<1%), yet how a biological system can fine-tune its synapses remains an open issue (Seung PNAS 1996,93:13339-44). Some models solved this by making neurons or dendrites bistable (Koulakov et al Nat Neurosci 2002,5:775-82; Goldman et al Cereb Cortex 2003,13:1185–95), in agreement with the hysteresis found in the tuning curves of PN.

(3) Bilateral dependency: Silencing of PN from one side impairs the functioning of the contralateral neurons in half of the oculomotor range (Aksay et al Nat Neurosci 2007,10:494-504). This led to suggest that the two sides of the system work as independent networks. Modeling work showed that proper coordination of the two networks is possible if individual neurons have high synaptic thresholds.

Here we investigate the construction of models that observe all these features, yet rely on standard single neurons – without the need for bistability or high synaptic thresholds. Using rate models, we study the class of networks which obey the recruitment order. Under these constraints both ipsilateral excitation and contralateral inhibition are necessary to maintain eye position. Interestingly, several of our models reproduce the inactivation experiments. We conclude that the inactivation result does not prove the independence of the two sides, but could be a simple consequence of the recruitment order of PN.

We solve the fine-tuning problem by assuming that neurons adapt their firing rates, a well-established biophysical process. The adaptation rule, based on (Moreau, Sontag Phys Rev E 2003,68:020901), leads to a network that is robust to perturbations in its parameters up to 5%, making the overall model more robust than fine-tuned network models. With this adaptation rule, hysteresis emerges as observed in the data, without resorting to hysteretic units. We therefore suggest that hysteresis is a signature of an active robustness mechanism, but not necessarily of a hidden bistability. We conclude by suggesting further experiments to test existing models.

ENHANCER-DRIVEN FLUORESCENT MEMBRANE MARKERS FOR STUDYING NEUROANATOMY IN *DROSOPHILA*

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How neurons are connected with each other and how they interact with other tissues are fundamental questions of neurobiology, as they are central to the understanding of how information is collected, relayed and processed. A key to studying neuronal connections or neuroanatomy is being able to label specifically individual or subsets of neurons *in vivo*. Many strategies have been developed to achieve this goal in the past. Among those, Gal4/UAS binary transcription system with a wide range of Gal4 choices has been the most successful way of neuronal labeling in *Drosophila*. However, with the genetic tools currently available, it is still difficult to simultaneously label two populations of neurons with different probes, or to manipulate gene expression in one cell type while labeling another cell population. We seek to solve this problem by labeling neurons with enhancer-driven fluorescent membrane markers. To make highly expressed bright fluorescent membrane markers suitable for live imaging of the extensive membrane surface of neurons, we tried to find an optimal construct design with *Drosophila* class IV da neuron as a test model. By systematically testing the effects of different core promoters, 5'UTRs, 3'UTR/polyA signals, and addition of introns and insulators on the expression level of a CD8-GFP reporter *in vivo*, we first identified a design that significantly enhances the expression level of transgenes compared to the conventional strategy. We then managed to enhance the brightness of the reporter by multiplying fluorescent proteins with tandem-dimer design and polycistronic designs based on 2A peptide and IRES. Finally, we improved the evenness of the reporter on the cell surface by utilizing a CD4 transmembrane domain. Based on these improvements, we made two vectors that allow for strong labeling of neuronal processes with either green or red membrane markers *in vivo*. By incorporating the Gateway system and ΦC31 site-specific integration system, these vectors are versatile and efficient in cloning neuronal enhancers and transforming *Drosophila*. To demonstrate the usability of these vectors, we show that subsets of neurons in the larval brain can be labeled with two different colors to study neuronal connections. In addition, for the first time, we show the interactions between class IV da neuron dendrites and epidermis in live animals by labeling dendrites and epidermis in different colors.

Cell type-based analysis of microRNA profiles in mouse neocortex

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Neocortical circuits consist of a rich array of functional units - diverse neuron types with stereotyped location, connectivity patterns, and physiological properties. Neuron identity and phenotypes are largely determined by the unique pattern of gene expression. microRNAs are ~22nt noncoding RNAs that regulate mRNA translation and stability in a sequence specific manner. Recent studies have shown that some miRNAs are expressed in a cell type and/or developmental stage specific manner and may be critical to the establishment and/or maintenance of cell identity. To systematically profile miRNA expression in different type of neuronal cells, we are establishing a cell type-based “miRNA-tagging” and affinity purification system using genetically engineered mice. Cre-LoxP knock-in system is used to express epitope tagged Agonaute 2(tAgo2) protein in specific cell type. Because Ago2 is a core RISC component associating with mature miRNAs, affinity purification of tAgo2 under appropriate conditions co-precipitates bound miRNA from a genetically defined cell type. Deep sequencing is used to profile the captured miRNAs. Using this method, we are profiling miRNAs in major types of neurons in the neocortex and cerebellum. These results will guide functional studies of miRNAs which contribute to cell identity, circuit development, plasticity, and disorder in the neuronal system.

Circuit-specific expression of channelrhodopsin restores visual function in blind *rd1*, *rd16*, and *rho* -/- mice

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Purpose: Channelrhodopsin-2 (ChR2) is a light-sensitive protein that, when expressed in neurons, depolarizes the cell in response to light stimulation. Microelectronic neural prostheses result in broad and indiscriminant stimulation of the neural interface, whereas expression of ChR2 can be genetically-targeted using cell type-specific regulatory sequences (i.e., promoters) such that activation of specific neural circuits can be achieved. Using the GRM6 regulatory sequence in combination with a tyrosine-mutated adeno-associated virus (AAV), we were able to target expression of ChR2 to the ON bipolar cells of the retina using either a subretinal or intravitreal injection, and subsequently restore visual function in multiple mouse models of blindness.
Methods: We evaluated retinal bipolar cell transduction using wild-type and capsid tyrosine-mutated AAV serotypes. Vector, including the ChR2 and green fluorescent protein (GFP) genes, was either subretinally or intravitreally injected in *rd1*, *rd16*, and *rho* -/- mice under the control of the GRM6 promoter. Expression and localization of the ChR2-GFP fused protein was evaluated using confocal microscopy and immunohistochemistry. Visual function was measured behaviorally in wild-type, untreated, and ChR2-treated mice using a water maze and retinal patch clamp recordings, respectively.
Results: Both wild-type and mutated serotypes were effective at transducing retinal bipolar cells. The capsid tyrosine-mutated serotypes were able to increase bipolar cell transduction by as much as 20-fold, even with an intravitreal injection. In the water maze task, the ChR2-treated mice learned the task nearly as well as the wild-type mice (the untreated mice were unable to learn the task). Additionally, the light intensity necessary to restore this visually-guided behavior was within the normal visual dynamic range of human vision.
Conclusions: Targeted expression of ChR2 in retinal ON bipolar cells restores circuit-specific computation, behavioral, and physiological visual function in all treated mice, suggesting the broad applicability of this gene therapy. Equally as important, we can target ChR2 expression to bipolar cells using a capsid tyrosine-mutated AAV, even with an intravitreal injection. Further research is necessary to evaluate visual acuity and methods of increasing the sensitivity in these treated animals, taking into account new variants of ChR2. Recent bioengineering work from our group in nonhuman primates suggests hope for a translational path.

Balance of excitation and inhibition in-vivo in auditory cortex of mouse models of autism

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Autism is a neurodevelopmental syndrome defined by a triad of symptoms: 1. impaired communication; 2. impaired social interaction; 3. restricted interests and repetitive or stereotyped behaviors. Despite the high prevalence of autism, its neural basis remains unclear. One theory postulates that autism arises from an imbalance between excitatory and inhibitory activity in the cerebral cortex. Supporting this theory is the high co-morbidity of autism with epilepsy, hyperactivity, or hypersensitivity to sensory stimuli, all of which suggest the presence of excessive excitation and/or deficient inhibition. An altered balance between excitation and inhibition has also been shown previously in-vitro, for example in neuronal cultures with disrupted function of neuroligins.

The auditory cortex is an excellent candidate for studying the relationship between excitation and inhibition synaptic input in vivo. Our laboratory has previously shown that excitation and inhibition are exquisitely balanced in the rat primary auditory cortex. The magnitude of excitatory and inhibitory conductances co-vary with sound frequency, and inhibition follows 3 ms after excitation in a stereotyped fashion. Moreover, auditory processing is often impaired in human autistic patients, many of whom show deficits in auditory discrimination, selective attention to localized sounds, etc.

To explore the hypothesis that disruption of autism candidate genes results in an imbalance of cortical excitation and inhibition, we studied this balance in three mouse models of autism. We used in vivo whole-cell patch-clamp recording technique to record sound-evoked subthreshold activity in single neurons in the primary auditory cortex of anesthetized mice (24-40 days old). Using previously described techniques, we decomposed evoked currents into their underlying excitatory and inhibitory components.

We found that in wild-type mice (C57Bl6, CBA, FVB), excitatory and inhibitory conductances were co-tuned, showing similar dependence on tone parameters in a given cell. Excitation was followed by inhibition after a brief (few milliseconds) delay. These results confirm that precise stereotypical sequence of excitation and inhibition described in rat auditory cortex is also observed in auditory cortex of wild-type mice.

Interestingly, we also observed the same stereotypical sequence of excitation and inhibition in-vivo in three different mouse models of autism: FMR1 (Fragile X mental retardation 1) knockout mouse, NL3 (Neuroligin 3) R451C knockin mouse, and MECP2 (Methyl CpG binding protein 2) knockout mouse. These results suggest that the imbalance between excitation and inhibition observed in-vitro may not be a general property of cortical responses, but may instead be limited to specific cell types, cortical layers, or cortical areas.

ANALYSIS OF NEURAL NETWORK BASED ON THE LINEAGE-DEPENDENT NEURAL CIRCUITS IN DROSOPHILA

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ANALYSIS OF NEURAL NETWORK BASED ON THE LINEAGE-DEPENDENT NEURAL CIRCUITS IN DROSOPHILA

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To identify all the neural circuits of the Drosophila brain systematically, we focused on the clonal units. A clonal unit is a set of characteristic neural circuits formed by the neurons that are derived from a single neuroblast. Theoretically, the cerebrum of the adult brain should consist of about 100 clonal units, because there are as many neuroblasts per hemisphere of the developing brain. By visualizing the neuroblasts and their progeny using the MARCM technique, we have so far identified in total 83 clonal units. Among them 70 units are newly identified, and 13 units are previously identified ones. We classified these clonal units into 5 groups according to their characteristic distribution of presynaptic sites. To record the complex projection patterns of each clonal unit systematically, we developed a formulation system of the neural projection patterns on the basis of a detailed brain region map. Analysis of the database of the identified clonal units provides information about neural communications between brain regions and suggests that the central complex and anterior ventrolateral protocerebrum might be the major cites that integrate information of different sensory modalities.

One channel, one vesicle; two channels, two vesicles: synaptic transmission at an analog synapse in the rod bipolar cell pathway of the mammalian retina

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The rod bipolar (RB) cell pathway of the mammalian retina conveys rod photoreceptor output to ganglion cells (GCs) under conditions in which ambient light intensity is low and photons are scarce (i.e., during scotopic vision). The RB cell pathway is a well characterized neural circuit: rods make synapses onto RBs, RBs make synapses onto AIIs, and AIIs are coupled electrically via gap junctions to ON cone bipolar cells (which in turn drive ON GCs) and make inhibitory glycinergic synapses onto some OFF cone bipolar cells and GCs. There is extensive convergence of rod outputs onto single GCs: a single GC can pool the output of up to 10,000 rods. This convergence makes GCs sensitive but raises the requirement for stringent noise-reducing and gain-controlling mechanisms within the RB pathway. Here we examined coupling between presynaptic Ca channel opening and coordinated multivesicular release (MVR) at ribbon synapses by making paired recordings from the pre- and postsynaptic neurons at the retinal rod bipolar (RB)-AII amacrine cell synapse. Evoked exocytosis was steeply dependent upon external Ca^{2+} concentration ($[Ca^{2+}]_e$) but varied linearly with the number of open Ca channels, consistent with a synapse at which $[Ca^{2+}]$ microdomains generated by the opening of single channels control the release of individual vesicles. By applying a slow voltage ramp to the presynaptic RB, we found that uniquantal synaptic events were evoked reliably at potentials sufficient to open only a single presynaptic Ca channel at an individual active zone (AZ). Raising $[Ca^{2+}]_e$ did not promote MVR at this threshold, though it did increase the probability of observing uniquantal events. A transition from uni- to coordinated multiquantal release occurred when the presynaptic membrane was depolarized sufficiently to allow multiple Ca channels to open at a single AZ. The appearance of MVR at potentials sufficient to open two Ca channels at a single AZ permits the RB to signal using MVR in a voltage regime in which Ca conductance increases linearly with membrane potential. This allows the synapse to amplify small presynaptic voltage changes near the threshold for exocytosis and to encode presynaptic membrane potential in a linear manner over a wide dynamic range.

How *Drosophila* Larvae Stay in the Dark

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Drosophila larvae exhibit negative phototaxis (light avoidance) during their first, second, and early third instar developmental stages. The light sensor for phototaxis is the Bolwig's Organ, a bilateral eye-like structure on the surface of its head. However, precisely how the larva uses sensory information acquired by the Bolwig's Organ to enact phototaxis has not yet been examined. Here, we use a tracking system to quantify the detailed movements of individual larvae as they respond to defined spatiotemporal patterns of luminosity. In regions of constant luminosity, a larva's trajectory is a sequence of runs (periods of forward movement) that are interrupted at random by abrupt turning events, during which the larva pauses and sweeps its head back and forth until it starts a new run in a new direction. Our analysis uncovers specific rules by which the larva regulates its movements in response to photosensory inputs, rules that yield trajectories in which the larva spends most of its time in the dark. These rules for navigating luminosity gradients provide insight into the underlying sensorimotor transformations that are carried out by neural circuits for phototaxis.

THE PROPORTION OF REGULAR-SPIKING AND BURSTING EFFERENTS FROM SUBICULUM VARIES ACCORDING TO THE TARGET REGION

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Pyramidal neurons in subiculum project to various areas in the brain such as amygdala, nucleus accumbens, entorhinal cortex and orbitofrontal cortex to convey information processed in hippocampus. Anatomical studies using double-labeling have demonstrated that each principal neuron in subiculum projects to a single target region. Our previous studies on subicular pyramidal neurons showed that two groups of subicular pyramidal neurons – bursting and regular spiking neurons – are distributed in an organized fashion, with more regular-spiking neurons close to the CA1 and more bursting neurons further away from the CA1. Since the firing patterns of the projecting neurons determine inputs relayed neurons to different target regions, establishing the relationship between the firing properties of projecting neurons and their target areas would help us understand functional importance of the dichotomy of the neurons in subiculum.
To determine the physiological properties of neurons projecting to a certain region, we injected fluorescent beads to one of nine different regions in the brain in vivo. The beads were retrogradely transported to the subiculum and we later conducted current-clamp recordings from the labeled subicular neurons in acute rat brain slices. We found that the neurons projecting to nucleus accumbens (NAc), amygdale (Amyg), medial/ventral orbitofrontal cortex (MO/VO), and lateral entorhinal cortex (LECx) were located primarily in the proximal subiculum while those to retrosplenial cortex (RsCx), ventromedial hypothalamus (VM Hypo), presubiculum (PreSUB), and medial entorhinal cortex (MECx) were found almost exclusively in the distal subiculum. The percentages of bursting neurons projecting to the first four regions (NAc, Amyg, MO/VO, and LECx) were lower (6-26 %) than in neurons projecting to the latter four regions (73-92 %), which is consistent with our previous observations concerning the distribution of regular spiking and bursting neurons along the proximal-distal axis of the subiculum. IAM-projecting neurons were located in the area between the proximal and the distal subiculum, with 44% of bursting neurons. Our results suggest that the firing properties of subicular pyramidal neurons are correlated to their position along the proximal-distal axis and their projection, implying that different target regions of the subiculum receive different amounts of regular-spiking versus bursting inputs.

Deciphering neuronal circuits in the embryonic chick spinal cord utilizing intersection between newly identified enhancer elements

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Employment of neuronal subtype enhancer elements to drive expression of reporter genes in neurons is a widely used paradigm for deciphering neuronal circuits. Several caveats hamper the use of enhancer elements for tracking axonal trajectories of spinal interneurons (IN): 1) There are few genes that are expressed only in a specific IN subpopulation, rather a combinatorial code of gene expression determines the neuronal fate of INs. 2) Only few IN enhancer elements were characterized. 3) For tracking axonal projection of IN in vertebrates, germ line-targeted reporter genes yield bilaterally-symmetric labeling. Therefore, it is hard to distinguish between the ipsi- and contra-laterally projecting axons.

Utilizing transient-transgenic chick screen of highly conserved non-coding sequences we have identified 10 enhancer elements that are expressed in spinal neuronal subpopulations. Two enhancers are specific to one IN while the other are expressed in two or more neuronal populations. Further targeting of specific IN was employed by molecular intersection between enhancers employing DNA site-specific recombinases: Cre, Flp, PhiC31 and the Gal4 transactivaor. To this end, tools for tracking the axonal projection and targeting of the dorsal interneurons populations: dI1, dI2 and dI3, were generated.

We found that each dI neuronal population has a unique axonal patterning that differs in the ipsi/contra-lateral, rostral/caudal and the ventral/dorsal axes, and specific neuronal targets. A transcriptional code distinguishes dI1-3 neurons. The expression of Lim-HD proteins Lhx2/9, Lhx1/5 and Isl1 is restricted to dI1, dI2 and dI3 neurons, respectively. To begin to understand the possible transcriptional control of Lim-HD on dI1-3 axonal projection, the Lim- HD code of dI neurons was altered by cell-specific ectopic expression. Our data points to an instructive role for Lim-HD in patterning the axonal trajectories of dI neurons. Lhx9, Lhx1 and Isl1, are sufficient to confer dI1, dI2 and dI3 axonal pattering, respectively.

Cell type specific optical stimulation reveals left-right asymmetry of hippocampal plasticity.

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Postsynaptic spines at the CA1-CA3 synapse differ in shape, size and glutamate receptor expression depending on the hemispheric origin of the CA3 afferent. To study functional consequences of this asymmetry, we used optogenetic tools to selectively stimulate CA3 pyramidal cell axons from either the left or right hippocampus *in-vitro*. We find that input from the right CA3 produces less long-term potentiation (LTP) at CA1 synapses than input from the left CA3.

We made stereotactic injections of an adeno-associated virus containing double-floxed inverted Channelrhodopsin2-EYFP (Sohal et al., Nature 2009) into either the left or right CA3 region in adult αCamKII-cre transgenic mice. This led to selective expression of Channelrhodopsin2-EYFP in αCamKII positive cells (i.e. excitatory cells) in the CA3 region of one hippocampus and their projections (Schaffer collateral and commissural fibers). Spot illumination with 473 nm laser light (42 mW mm^{-2}) in the stratum radiatum of CA1 in hippocampal slices prepared from these mice 8-10 days after viral injection generated reliable excitatory postsynaptic responses in whole cell-recorded CA1 pyramidal cells. To investigate LTP at these synapses, we applied a burst pairing protocol (Pike et al., J Physiol 1999) using light stimulation to activate axons originating from the CA3 of one hemisphere only. We found a significant difference in timing-dependent LTP (t-LTP) between the two sides:

In slices from left CA3-injected animals, we found that this pairing protocol reliably elicited t-LTP in CA1 pyramidal cells recorded both ipsi- and contralaterally (ipsi: electric: 156 ± 9%; light: 143 ± 7%, n=6; contra: electric: 166 ± 27%; light: 171 ± 23%, n=6). In contrast, in slices from right CA3 injected mice, the light evoked input did not yield any significant t-LTP (ipsi: 100 ± 9%, n=6; contra: 105 ± 8%, n=6), whilst the electrical stimulation induced LTP (ipsi: 156 ± 16%, n=6; contra: 163 ±15%, n=6).

Our results suggest that hippocampal computations in adult mice might be more lateralised than previously thought.

CEREBELLAR-PROJECTING NEURONS SERVE AS LINEAR FILTERS OF INFORMATION ENTERING THE CEREBELLUM

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The cerebellum integrates sensory, motor and cortical information to calibrate movements. Cerebellar-projecting neurons (CPNs) convey these signals to the cerebellum via mossy fibers and are critically important for proper functioning of the cerebellum. However, little is known about how CPNs filter information and thus contribute to cerebellar computations. Whether CPNs faithfully relay their inputs with little transformation or perform more complex computations is unknown. In this study, we examine the intrinsic filtering properties of CPNs to determine how they transform somatic input into firing rate output.

To identify neurons projecting to the cerebellum, we performed texas red dextran injections into the mouse cerebellum and targeted whole-cell patch-clamp recordings to retrogradely labeled neurons in eight precerebellar nuclei in pontine and brainstem slices. Though these nuclei convey diverse information to the cerebellum, the transformation of input current into firing rate was remarkably similar across neurons. CPNs in all areas responded to somatic depolarizing pulses with a linear or bilinear current-to-firing rate relationship over a very wide range of input currents and firing rates. When probed with sinusoidally modulated current, CPNs responded by modulating firing rate in phase with the input and with constant gain across input frequency. Therefore, although CPNs are an essential component of cerebellar circuits, they do not intrinsically contribute to complex signal processing; rather, they serve to linearly scale the inputs that reach their soma into signals within an appropriate range for computations subserved by the cerebellar cortex.

THE ESCAPE NETWORK MAPS ONTO THE TRANSMITTER/TRANSCRIPTION FACTOR STRIPE PATTERNING IN THE HINDBRAIN OF ZEBRAFISH

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We are exploring the possibility that there is a common ground plan shared by many motor networks throughout hindbrain and spinal cord. Our previous anatomical studies of larval zebrafish revealed that there is a novel organization in hindbrain where alternating stripes of inhibitory and excitatory interneurons span multiple segments containing different sensorimotor circuits. Within each stripe, neurons share axonal projection patterns and transcription factor codes, with the earliest born neurons located ventrally and the latest dorsally. To begin to examine how particular networks in hindbrain map onto the stripe organization, we examined neurons contributing to the escape circuit, one of the earliest born circuits, in larval zebrafish. We identified 4 types of interneurons that cause excitatory or inhibitory inputs to Mauthner (M) cell, which plays a major role in the initiation of escape behavior: 1) Feedforward inhibitory neurons receive excitatory inputs from auditory or tactile inputs and send their inhibitory inputs to M cells. They were located in the ventral end of the lateral glycine stripe and had the bilateral projections characteristic of the lateral glycine stripe. 2) Feedback inhibitory neurons fire in response to M cell's action potential and feed inhibitory inputs back to M cell. They were located in the ventral end of the medial glycine stripe and had the primarily ascending and purely ipsilateral projections characteristic of neurons in the medial glycine stripe. 3) Cranial relay neurons are excitatory cells that fire in response to the M cell's activation and cause inhibitory input to the M cell via their excitation of feedback inhibitory neurons. They were located ventrally in the region of the middle stripes and had primarily contralateral projections, characteristic of the middle stripes. 4) Spiral fiber neurons are excitatory cells that have excitatory inputs to the contralateral M cell. They were located ventrally in the region of the middle stripes and had the primarily contralateral innervations characteristic of the middle stripes. Taken together, all of the known interneurons involved in the earliest developing escape circuit are located in the ventral end of stripes amongst the earliest born cells, consistent with other evidence that the age axis of stripes maps onto function with the oldest ventral neurons controlling fast behaviors and the youngest driving slower ones. The neurons in the escape network also exhibit the axonal projection pattern we would predict from our earlier work based upon their stripe location. Our data indicate that an individual network draws components from stripes that are patterned by cell type, age, and functional role. This pattern may form a foundation for neuronal circuits throughout hindbrain (and spinal cord) involved in a wide variety of sensorimotor processing.

Alterations in cortical GABA inhibitory circuits in mouse model of Rett Syndrome

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Rett syndrome (RTT) is a neurodevelopmental disorder caused by mutations in the X-linked gene encoding methyl CpG binding protein 2 (MeCP2). MeCP2 has been implicated in chromatin remodeling, DNA binding and transcription regulation, and modulation of RNA splicing. RTT is hypothesized to result from inappropriate synaptic connectivity and/or plasticity, but it remains largely unclear which synaptic circuits are affected and how cellular impairments relate to the onset and progression of the disease. Until recently, efforts have been almost exclusively directed towards studying the effects of MeCP2 mutations on glutamatergic excitatory neurons. Here we examine the impact of MeCP2 deficiency on the development and function of GABAergic inhibitory circuits.
In most brain regions, GAD67 is the rate-limiting GABA synthetic enzyme. GAD67 activity is in part at the level of transcription, which is regulated during development, and by neural activity and experience. GABA signaling not only mediates inhibitory transmission but also regulates activity-dependent maturation of inhibitory synapses and innervation patterns. Therefore, alteration of *Gad67* transcription likely influences not only inhibitory output but also the development of GABAergic innervation. In order to determine the impact of MeCP2 deficiency on *Gad67* transcription with cell type resolution *in vivo*, we generated a knockin reporter strain in which d2GFP is co-expressed with GAD67 (GAD67-D2GFP). By analyzing GAD67-d2GFP; MeCP2 null male mice, we found *increased* transcription of *Gad67* in cortical layers as early as P14. This increased expression likely contributes to the enhanced inhibitory drive that has been observed in layer 5 pyramidal neurons. Most of these GAD67+ cells belonged to the parvalbumin-expressing (PV) class of fast-spiking basket cells, which innervate the perisomatic region of pyramidal neurons and control their output and synchrony. However, neuropeptide Y and somatostatin (markers for other subtypes of GABAergic neurons) expression was decreased in the processes of cortical and striatal neurons of P30 MeCP2-null males. These data suggest that MeCP2 deficiency affects GABAergic neurons and consequently, the network, in cell type-, brain region-, and developmental stage- specific manner. We are currently examining the impact of altered MeCP2 expression on the maturation, innervation pattern, and physiology of subtypes of GABAergic neurons *in vivo*. These studies will significantly improve our understanding of how altered MeCP2 expression disturbs the development of GABAergic circuits and reveal new insights into the pathogenesis of RTT.

Genetic dissection of amygdala neuronal circuitry for fear and anxiety in mice

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Despite the fact that the role of amygdala for fear, anxiety and related disorders is well established by various conventional methods, the structure and function of neuronal circuits that control these behaviors are not well understood. In our lab, we have combined mice genetics with the recently developed genetic techniques of manipulating neuronal activities to identify neuronal populations in the amygdala and link them convincingly to appropriate behavioral responses. In addition to this approach, we are using genetic methods of anterograde and retrograde tracing so as to define the structure and function of the fear circuits. As an entry point, we have identified a neuronal population (labeled by a gene expressing PKC-delta) in the main fear output region called central amygdala (CeA). We show that this inhibitory neuronal population negatively regulates learned fear using a pharmaco-genetic silencing approach. These interesting data from these experiments will be presented in this meeting.

Modeling self-sustained firing in motoneuron after spinal cord injury

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Motoneurons have the ability to generate plateau potentials resulting in self-sustained firing and providing a mechanism to translate short lasting synaptic inputs into long lasting motor output. During the acute stage of spinal cord injury (SCI) the endogenous ability to generate plateaus is lost; however, during the chronic stage the plateau potentials reappear. We extend previous modeling studies of the physiological changes in spinal motoneurons following SCI in order to investigate mechanisms that might contribute to spasticity due to the self-sustained firing. Experimental data are used to determine the parameters of the two compartment conductance based differential equation model for SCI.

Using mathematical analysis and simulation results, we study the effect of changes in kinetics and morphology on motoneuron excitability. Our modeling studies show that changes in morphology following SCI like increase in soma size and decrease in dendritic arbor do not contribute to increases in self-sustained firing. Increases in self-sustained firing are likely to occur due to changes in membrane conductances and changes in synaptic activity, particularly changes in the strength and timing of inhibition.

IDENTIFICATION AND ELECTROPHYSIOLOGICAL RECORDINGS OF MEDIAL PREFRONTAL CORTICAL INTERNEURON SUBTYPES IN BEHAVING MICE

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Neural circuits exhibit exquisitely precise connections between specific, often molecularly defined, neural cell types. Genetically targeted fluorescent markers provide a powerful handle to explore the specificity in the architecture of neural circuits, mostly using in vitro preparations. In contrast, successes in systems neuroscience have come mainly from relating activity of single neurons with perception and behavior, without explicit knowledge of the identity of recorded neurons. Our goal was to bridge this gap by recording from molecularly identified neurons during behavior.
We first made defined neural classes light-activatable by expressing ChannelRhodopsin-2 (ChR2). We selected two populations with distinct connectivity patterns: parvalbumin (Pv) interneurons that target pyramidal cells and calretinn (Cr) interneurons that target other interneurons. Next, we used a CMV-loxpSTOPloxp-ChR2 AAV vector to express ChR2 in these neurons. We observed highly specific (~95%) and efficient (~50%) expression of ChR2 in both PV and Cr neurons in the medial prefrontal cortex.
To record in behaving mice we designed a light-weight microdrive (~4g) housing up to 10 independently movable tetrodes and optical fibers. We also built miniaturized fiberoptic connectors mounted on the drive to pass light from an external laser. Using these tools we are able to both stimulate and record neurons in freely moving mice.
We found that short light pulses (1-5ms) reliably evoke action potentials (APs) in a subset of neurons and cause inhibition in others. Pv+ neurons could be reliably driven to over 200Hz. Local field potentials (LFP) showed light-induced positive deflections and a long-lasting rebound (100-150ms). While the light-induced effects observed in well-isolated single units did not substantially outlast the duration of the light pulse, sufficiently powerful light pulses (>100 mW/mm2) generated a photoelectric effect with multiple timescales that did outlast the stimulus, making it difficult to interpret LFPs. We hypothesized that the heterogeneity of prefrontal cortical responses is accounted for by neural type, and therefore sought to characterize the behavioral correlates of identified units. For well-isolated units the shape of the light-evoked APs was identical to physiological ones, allowing us to "optically tag" neurons. Previous studies relied on AP shape to identify neurons. Indeed, we found that most Pv+ neurons had narrow APs, but there were also some exceptions. Therefore we used these techniques to record identified Pv+ and Cr+ from medial prefrontal cortex. We are presently characterizing the behavioral correlates of these two neural populations.

Differential impact of GAD67 deficiency on inhibitory synaptic transmission in primary visual vs. medial prefrontal cortex in mice.

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A highly reproducible molecular pathology in schizophrenia (SZ) is the reduction of mRNA for GAD67, the rate-limiting enzyme for GABA synthesis, in subtypes of cortical inhibitory interneurons. This is particularly apparent in parvalbumin (PV) expressing interneurons. PV+ cells play an important role in the generation and maintenance of gamma range oscillations, which are implicated in the dynamic organization of cell assemblies. Dysfunction in PV+ cells is thought to underlie certain aspects of cognitive deficits in SZ, such as working memory impairment. The functional impact of GAD67 deficiency in neocortex is unclear.

To model the cell-type specific reduction of GAD67 observed in SZ, we used the cre/loxp system in transgenic mice to conditionally knockdown GAD67 in PV+ cells. Viral-vector mediated GFP expression in a PV-Cre mouse line was used to identify PV+ cells for electrophysiological analysis. GAD67 reduction led to faster IPSC decay in paired recordings (PV+ to pyramidal cell connections) from two different cortical regions: Medial prefrontal cortex (mPFC) and primary visual cortex (V1). Faster decay appears to be the direct result of reduced synaptic GABA concentration. Surprisingly, knockdown of one allele of GAD67 led to a greater than 50% reduction of IPSC amplitude in mPFC, but resulted in no change in IPSC amplitude in V1.

Cells and circuits in specific cortical regions are differentially sensitive to GAD67 reduction in PV+ cells. In particular, PFC circuitry appears to be more vulnerable to GAD67 deficiency. Further understanding of the function of GAD67 in inhibitory transmission and network dynamics will inform future experimental and modeling studies focused on the role of GABA interneurons in schizophrenia.

The contribution of active dendritic properties to memory dynamics in a network

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Active properties in the dendrites of several neuronal cell types have been found to enable nonlinear computations that play a large role in a single neuron's input-output relationship and its response to network inputs. Here we focus on understanding how these nonlinear properties may contribute to the generation of short-term memory in a network. We have extended a theoretical framework for studying short-term memory networks to incorporate biophysically realistic conductances in multiple dendritic compartments. We find that this network can be tuned to as many stable activity levels as there are dendritic compartments; with realistic parameters, this leads to ~1 Hz gradations in output rate, consistent with the smooth, continuous encoding seen in experimental data. Further, the network shows insensitivity to simulated lesions where connectivity parameters are varied by <10%, exhibiting robustness in combination with graded persistent activity. In correlation to its robustness, the network is insensitive to weak stimuli; however the degree of sensitivity can be controlled by the addition of synaptic noise, making the system sensitive to small, low-frequency signals.
To test for these contributions, we have developed methods for imaging calcium activity in dendritic branchlets during behavior. Using somatic electroporation, we loaded single cells in the larval zebrafish with calcium sensitive dyes. Individual branches are visible within minutes with enough intensity to allow stable, long-term monitoring of activity. Localized glutamate delivery can be used to stimulate targeted branches, evoking fluorescence changes of up to 50%. Together, these theoretical and experimental approaches will open up ways to relate activity in individual branches or compartments along a dendrite with cellular and network function, as well as behavior.

Spatiotemporal control of neural activity in freely behaving *C. elegans*

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We demonstrate an optogenetic illumination system capable of stimulating or inhibiting neurons or muscle in arbitrary spatiotemporal patterns in freely behaving *C. elegans*. A tracking microscope with a video camera records the posture and motion of an unrestrained worm expressing Channelrhodopsin-2 (ChR2) and/or Halorhodopsin (Halo) in specific tissues. Custom image processing software analyzes the worm's position and rapidly estimates the locations of targeted muscles and neurons. The software then directs a digital mirror device to illuminate the targeted cells with laser beams of the appropriate wavelengths. Since each neuron in a behaving worm is a moving target, speed is of the essence. Our system operates in real time and analyzes a 1024 x 768 pixel image of the worm in less than 10 ms. We use the system to perturb activity in the worm's neural circuits and quantify the resulting behavioral output. In particular, we demonstrate the use of this system to draw functional maps of the *C. elegans* mechanosensory neural circuit. We discuss potential extensions of our device to other model organisms.

$GABA_A$-mediated excitation in CA1 pyramidal neurons: A computational study.

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Traditionally, a decrease in GABAergic activity is thought to underlie seizures, yet almost one-third of epileptic patients do not respond to medications which augment GABA activity. In the CA1 region of the hippocampus, prolonged high-frequency stimulation in vitro of GABAergic (inhibitory) interneurons induces seizure-like network activity. Potentially underlying this seizure-like activity, pyramidal cells that receive GABAergic stimulation from these interneurons exhibit paradoxical burst discharge. Thus, the interneuron-pyramidal cell network may transiently become a recurrent excitatory network. There are two primary mechanisms that may contribute to this GABA mediated excitation: intracellular chloride accumulation, and increased extracellular potassium concentration. Here we test the necessity of each. We use a computational model of the CA1 pyramidal cell to study the generation of GABA-mediated excitation. The model incorporates the detailed morphology of a CA1 pyramidal cell, $GABA_A$-receptor density gradients, dynamic ion concentrations, an extracellular space with glial buffering of potassium, and ion transport mechanisms. In the model, high frequency stimulation of a small fraction of $GABA_A$ receptors leads to a GABA-mediated depolarization with kinetics and magnitude comparable to experimental recordings. The depolarization in the model results from the accumulation of chloride inside the neuron that exceeds the transport capacity of the potassium chloride co-transporter KCC2, regardless of the extent of potassium accumulation in the extracellular space. Introducing a physiological potassium transient alone under similar conditions does not lead to burst discharge. The model supports the hypothesis that chloride accumulation through the $GABA_A$ receptors is sufficient to lead to GABA-mediated depolarization, and does not require extracellular potassium transients. The transient chloride accumulation may be a mechanism for switching the pyramidal-interneuron network from excitatory-inhibitory coupling to recurrent excitatory coupling, potentially contributing to the generation of epileptiform activity.

Postnatal maturation of Parvalbumin interneuron-mediated inhibition in primary visual cortex

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Neuronal circuits often display remarkable plasticity to sensory input especially during early postnatal life. A representative example is that the closure of one eye during a critical period can permanently shift the response property of neurons in the primary visual cortex (V1) to favor inputs from the open eye – ocular dominance (OD) shift. Although much progress has been made in studying the anatomical, physiological and molecular components of OD plasticity, a comprehensive understanding that integrate these components in the context of relevant cortical circuitry remains a daunting challenge. Accumulating evidence suggests that proper function of GABAergic inhibitory neurons in V1 are critical to establish the physiological circuit architecture that allows OD plasticity to proceed. Indeed, deficiency in the GAD65 (isoform of GABA synthetic enzyme) abolishes OD plasticity, while activation of α1 subunit-containing GABAA receptors (α1-GABAARs) triggers OD plasticity. Recently, a subset of inhibitory neurons – Parvalbumin (Pv) positive basket cells – is suggested to be involved in OD plasticity. However, all evidences so far are correlative and inconclusive.

Combinging cell type-specific Cre knockin mice, Cre-activated viral gene expression and electrophysiology, we systematically examined the development changes of V1 Pv cell network around the critical period (from postnatal 17 to 44). These include: (1) intrinsic properties of Pv cells, (2) firing features of Pv cells, and especially (3) Pv cell mediated GABAerigc synapses on both pyramidal cells (Pv-Py) and Pv cells (Pv-Pv). We found that the changes in both intrinsic properties and firing features of Pv cells support the developmental enhancement of the typical fast-spiking characteristics during this period.
As for the Pv cell mediated inhibition, both Pv-Pv and Pv-Py synapses exhibit very similar short term plasticity with slight developmental changes. Interestingly, Pv-Pv synapses always show much faster kinetics than Pv-Py synapses at different development stages. But both types of synapses mature with increasingly faster kinetics with the same developmental trajectory before postnatal 23, and then become stable. This maturation time course correlates with the developmental increase of α1-GABAARs. Such kinetics change is also specific at Pv cell mediated synapses. Both the time course and the specificity suggest that the maturation of Pv cell mediated inhibition is involved in the onset of critical period in primary visual cortex.

A Gut Peptide Receptor is Selectively Expressed on Midbrain Dopamine Neurons and Mediates Behavior via Novel Physiological Mechanisms

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Midbrain dopamine neurons in mammals regulate a diverse array of important behavioral processes and their deficits are associated with several human psychiatric disorders such as ADHD and schizophrenia. Thus it is highly valuable to identify cellular targets that selectively regulate the activity of these neurons. Here, we report that these neurons selectively express guanylyl cyclase-C (GC-C), a membrane receptor that had been believed to be specific to intestinal mucosal cells. GC-C knockout mice exhibit hyperactivity and attention deficits, indicating that GC-C plays important role in behavioral control. Activating GC-C by two gut peptide ligands potentiates the excitatory effects mediated by the neurotransmitters glutamate and acetylcholine via the activity of cGMP-stimulated protein kinase (PKG). Our results reveal a surprising role of membrane guanylyl cyclase in behavioral regulations and suggest that GC-C and its downstream signaling components may provide valuable therapeutic targets for treating psychiatric disorders.

AXON BRANCHING IN THE DEVELOPMENT OF SPINAL SENSORY CIRCUITS

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The spinal sensory circuits are constructed by many different types of axonal branches that are important for transmitting somatosensory information. The central afferents bifurcate at the dorsal spinal cord and generate two branches that extend in opposite directions along the rostrocaudal axis. These ascending and descending projections then sprout interstitial collateral branches that project ventrally to make mono- or poly-synaptic reflex connections. To understand the development of these branches, we identified an extracellular cue that regulates bifurcation of sensory afferents. It involves the C-type natriuretic peptide (CNP), which is related to the atrial natriuretic peptide that regulates water secretion in the kidney and is highly expressed in the dorsal spinal cord. CNP binds to the membrane receptor Npr2 and stimulates the production of cGMP. When treated with CNP or pharmacological agents activating the cGMP pathway, cultured neurons generate exuberant axonal branches. More importantly, genetic deletion of the hormone or the cGMP-dependent protein kinase 1 results the failure of sensory axon bifurcation. Although collaterals are still formed from the remaining afferents, this defect leads to the loss of half of the sensory connections with the reflex circuits. Interestingly however, these mutant mice are viable and have no gross behavioral defects, despite that the bifurcation defect persists in the adult animals. These observations suggest some interesting features underlying the development of reflex circuits: 1) the amount of sensory inputs at the early stage has no influence on the mature reflex connections; 2) the normal reflex development is plastic enough so the loss of sensory inputs is compensated. Preliminary data from our detailed anatomical and physiological analysis of these mice will be presented to test these ideas.

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TARGETING SINGLE NEURONAL NETWORKS FOR FUNCTIONAL ANALYSIS *IN VIVO*

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Neurons in visual cortex show characteristic patterns of activity in response to specific features of a visual stimulus, including orientation, direction and spatial frequency. This stereotyped response, or tuning, arises from a transformation a neuron performs on its specific inputs in time. However, little is known about whether neurons that are synaptically connected share stereotyped tuning properties, or derive their own visual tuning properties from a combination of varying sharply and broadly tuned inputs. Here we present progress on the development of a novel strategy to record the activity of a single neuron and a subset of its monosynaptic inputs simultaneously, in relation to visual stimuli, in the living rodent. The method relies on combining a single cell tracing strategy with *in vivo* two-photon calcium imaging. Single cell electroporation of TVA (receptor for EnvA) and rabies glycoprotein targets the specific infection and complements the monosynaptic, retrograde spread of an EnvA pseudotyped, G-deleted rabies virus from a single neuron. By including the gene for GFP in the electroporation transfection and mCherry in the rabies genome, the postsynaptic neuron and presynaptic neurons are labeled uniquely. We show that this strategy is robust and reliable at labeling a single neuron and at least dozens of its presynaptic inputs in over one third of electroporation attempts in living mice. This labeling may be used to guide functional analyses of single synaptically connected neuronal networks. We demonstrate the ability to determine visual tuning properties, including orientation tuning, from Oregon Green Bapta-1 AM loaded electroporated and rabies infected neurons in chronic surgery preparations. We describe the surgical and methodological parameters used to achieve adequate loading and imaging clarity to perform these experiments. We elaborate on applications of this approach to test the correlation between stereotyped visual responses and synaptic connectivity in single neuronal networks *in vivo*. These analyses may offer crucial insights into the computations a single neuron performs to determine its stereotyped pattern of activity in response to visual stimuli.

NOVEL EXPERIENCE IN ADOLESCENT MICE PROMOTES THE INNERVATION OF THE PREFRONTAL CORTEX BY VENTRAL TEGMENTUM AXONS

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The prefrontal dopaminergic system plays an important role in goal-directed behaviors, and dysfunctions in this system are implicated in major psychiatric disorders. Anatomically, the prefrontal dopaminergic axons arise from neurons located in the midbrain ventral tegmental area (VTA). Although an overall higher density of dopaminergic innervation has been observed in adults compared to young animals, the factors that influence the growth dynamics of the axon terminals are not known. To determine the effects of developmental age and behavioral experience on the formation of these terminals, we used anterograde tracer labeling and in-vivo two-photon microscopy to image the dynamics of VTA axon terminals in the prefrontal cortex and examined the effect of novel physical activity on the dynamics of these terminals in adults and juvenile mice. Here we show that there was no significant difference in the formation of VTA terminals in the home cage conditions in both adult and young animals. However, a significant increase in the formation of new terminals occurred in response to novel physical activity in the juvenile animals, but not in the adult animals. Our results suggest that the patterning of VTA terminals in the prefrontal cortex is jointly regulated by developmental age and behavioral experience. Moreover, individual episodes of novel experiences in adolescent animals are particularly effective in promoting the VTA innervation to the prefrontal cortex.

VISUALIZING NEURAL CIRCUITS WITH ACTIVITY-DEPENDENT NUCLEAR IMPORT OF A TRANSCRIPTION FACTOR

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Marking active neuron in behaving animals is necessary to bridge between behaviors and in vitro analysis of circuit function. Here we present a novel activity reporter system dubbed CaLexA (calcium dependent nuclear import of LexA) based on the mechanism of activity-dependent nuclear import of a transcription factor. Nuclear factor of activated T-Cell (NFAT) is a calcium responsive transcription factor. The transport of NFAT between the nucleus and cytoplasm is regulated by calcium-dependent phosphatase calcineurin. The basic strategy of the CaLexA system is to express an exogenous transcription factor in specific neuron populations and its import into the nucleus upon sustained depolarization induces the expression of the reporter gene. We employed two binary expression systems: the Gal4/UAS system for expressing the chimeric transcription factor LexA-VP16-NFAT, and the LexA/LexAop system for expressing the GFP reporter. We have tested this strategy on the well-studied odorant receptor neurons (ORNs) and projection neurons (PNs) in the *Drosophila* olfactory system. Our initial results using *Drosophila* antennal lobe show that exposure to physiological concentrations of fly pheromone generates high level expression of GFP in the appropriate glomeruli. In this proof-of-concept experiment using CaLexA, we restricted expression of the transcription factor to only ORNs or PNs and asked which neurons selectively respond to the pheromones. Our CaLexA system appears to have improved selectivity and signal-to-noise ratio over several similar systems using the immediate early genes. This approach validated in *Drosophila* should, in principle, be applicable to other model organisms with some modifications.

Optogenetic probing of cortical synaptic circuits measured through *in vivo* whole-cell recordings in the mouse barrel cortex

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Abstract: Channelrhodopsin-2 (ChR2) encodes a light-activated cation channel that can be used to gain optical control of genetically-defined neural circuits with millisecond precision. Here, we express ChR2 in neocortical excitatory pyramidal neurons located in the C2 column of the mouse barrel cortex. Using in vivo whole-cell recordings, we analyse the membrane potential changes evoked by brief blue light flashes. We find two classes of responding neurons. Putative ChR2 expressing neurons depolarise with very short latencies (217±48 μs, n=35). Precisely timed action potentials can be evoked reliably and these neurons therefore correspond to our genetically defined population of presynaptic neurons that we optically stimulate. The other class of neurons depolarise with much longer latencies (3.54±1.47 ms, n=57) indicating that they do not express ChR2. Their response must therefore result from the postsynaptic action of synaptically released neurotransmitters. Interestingly, the postsynaptic response is highly variable, despite the fact that presynaptic action potentials are reliably evoked. A large part of this postsynaptic variability can be accounted for by examining the pre-stimulus spontaneous fluctuations in membrane potential. Large depolarising light-evoked responses are recorded during a spontaneously hyperpolarised period (DOWN-state). However, when the neocortex is spontaneously depolarised (UP-state) the light-evoked response had only a small, brief depolarisation followed by a prominent hyperpolarisation. Somatic input resistance is not strongly affected by spontaneous activity and is similar in UP and DOWN states. By injecting current into the recorded neurons, we found that more inhibition was recruited when the stimulus was delivered in the UP state. These experiments provide insight into the response of the cortical neuronal network evoked by small excitatory perturbations induced by an optogenetic stimulus. Our results clearly indicate that a light-evoked stimulus of excitatory pyramidal neurons expressing ChR2 drives a prominent feedback inhibitory loop. This inhibitory feedback is likely to be mediated by local GABAergic neurons and may be of critical importance for balancing excitation and inhibition in the neocortex.

Motor Circuit Dysfunction in a *Drosophila* Model of Spinal Muscular Atrophy

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Spinal Muscular Atrophy (SMA) is the second most common autosomal recessive genetic disease in humans and is the most common genetic cause of infant mortality. SMA is caused by reduced levels of the Survival of Motor Neuron (SMN) protein, a component of a macromolecular complex that is required for the assembly of small nuclear ribonucleoproteins, essential components of the pre-mRNA splicing machinery.

We have analyzed Drosophila mutants of SMN and found that while both muscle size and locomotor activity are significantly impaired, neurotransmitter release at the neuromuscular junction (NMJ) is surprisingly increased. Furthermore, by inhibition or rescue of SMN in specific neuronal types in the motor circuit, we have found that this increase is not due to a requirement for SMN in motor neurons themselves, but instead is elicited by defective synaptic input from cholinergic interneurons which in turn induces motor neuron hyperexcitability. We have further found that inhibition of K+ channels in the cholinergic interneurons of SMN mutants or treatment with K+ channel pharmacological antagonists can restore normal NMJ neurotransmission in SMN mutants.

To identify molecules disrupted in the motor circuits of SMN mutants, we have carried out a screen for genes with defective pre-mRNA splicing. From this effort, we have identified a novel evolutionarily conserved transmembrane protein, stasimon, with reduced expression in SMN mutants. We show that restoration of normal levels of this protein in the cholinergic interneurons of SMN mutants reinstates normal neurotransmitter release from motor neurons. Our data reveals that cholinergic interneurons are an essential cellular site of action for SMN in Drosophila and show that restoration of normal physiological motor circuit activity ameliorates many deficits in this model of Spinal Muscular Atrophy.

INTEGRATION OF VISUAL AND PROPRIOCEPTIVE INFORMATION FOR REACHING IN MULTIPLE PARIETAL AREAS.

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When reaching to swat a mosquito on ones arm, a person may rely exclusively on somatosensory information to target the movement, or may also look at the arm to gather additional sensory information and improve his chances of squashing the mosquito. Many psychophysical studies show that humans optimally integrate sensory information, but little is known about how this integration is carried out in the brain.

The purpose of this study was to elucidate the neural mechanisms of integration by studying cortical responses in two rhesus macaques during reaches to an array of visual (VIS), proprioceptive (ipsilateral hand, PROP) or visual and proprioceptive (VIS+PROP) targets. Both monkeys showed reduced reach endpoint variability in the VIS+PROP task compared to the unimodal VIS or PROP tasks. Reduced variability is a behavioral hallmark of sensory integration, and neural models of integration suggest that it is achieved through enhanced neural responses to bimodal stimuli. Parietal Area 5 shows enhancement of responses to static proprioceptive position signals with vision of a realistic monkey arm, supporting the idea that the posterior parietal cortex plays an important role in sensory integration. We set out to characterize the integration of visual and proprioceptive signals in this region, specifically, Area 5 and MIP, during reach planning.

We found that many cells responded during multiple tasks with similar spatial tuning across tasks, though the degree of modulation was typically task-dependent. This finding contrasts with observations in other cortical areas (e.g., MST, VIP) and allows us to assess how response magnitude changes during integration with minimal complications due to differences in spatial tuning across tasks. Unimodal task preferences were heterogeneous in Area 5 and MIP. We first compared plan-related (delay period) activity of each neuron in the bimodal task to its best unimodal response. In Area 5 and MIP, preferred target responses displayed both enhancement and suppression (similar to other cortical studies of integration), with few of the additive or super-additive effects seen in superior colliculus. In contrast, the least-preferred target responses showed suppression of activity. These findings may be explained by response normalization or by sharpening of tuning curves in the bimodal condition, but are inconsistent with simple additive or enhancement models of integration. Further, neurons showed suppression across all target locations during the the movement period. These results challenge a simple additive or enhancement model of sensory integration. An understanding of the mechanism of sensory integration must account for the suppressive effects that we and others have observed, as well as the heterogeneity of response patterns across areas and across time. In particular, these results suggest that the process of integration may be better understood at level of the larger cortical circuit.

Mapping shared and unique components of neural circuits underlying different behaviors in *Drosophila*

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An animal's behavior is composed of a series of movements, drawn from a shared vocabulary of motor actions that can be used in multiple behavioral contexts. This flexibility hints at convergence of neural circuits on motor outputs for the execution of behavior. At what point in a circuit does this convergence occur? In the fly *Drosophila melanogaster*, it is largely unknown how many layers of interneurons might exist in a behavioral circuit, with the simplest proposal being that some sensory neurons may converge directly on motor neurons. Here, we identify novel neurons that are neither sensory nor motor, yet control the motor action of proboscis extension in the specific context of hunger but not thirst, providing a window into a segregated behavioral circuit. We describe quantitative assays for proboscis extension and genetic reagents for the manipulation of neuronal activity and the anatomical mapping of circuitry. By silencing or activating neurons (with transgenes such as *TrpA1, channelrhodopsin* and *Shibire*^{ts1}), we have screened for sets of neurons that are necessary and sufficient for the motor action of proboscis extension, in different behavioral contexts. These screens are providing a more detailed picture of the motor circuitry than was previously available, and revealing novel players in these circuits beyond known sensory inputs and motor outputs. We are characterizing these neurons and their involvement in multiple behaviors. Identifying the separate behavioral circuits that these neurons participate in will reveal the extent to which behaviors make use of dedicated circuitry, or potentially also share upstream command circuitry to trigger whole behavioral routines.

DISTRIBUTED PERSISTENCE DECAY TIMES IN A MODEL NEURAL INTEGRATOR

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Graded persistent activity encodes eye position in neurons of the horizontal velocity-to-position oculomotor neural integrator. In order to study the mechanisms of persistent neural activity, we have developed a preparation for synchronous eye tracking and two-photon laser scanning fluorescence imaging during spontaneous eye movement in the larval zebrafish. Within the caudal hindbrain of a larva bolus-loaded with the calcium-sensitive fluorescent dye Oregon Green BAPTA-1 AM, many neurons demonstrating eye position-correlated calcium fluctuations can be localized. Exponential fits to contraversive saccade-triggered average cellular fluorescence responses were used to estimate calcium impulse response functions. Ipsiversive saccade-triggered average fluorescence responses were deconvolved with these functions in order to reflect firing rate and then fit with exponential functions, the time constants of which measure activity persistence in between saccades. These time constants vary across at least 1-2 orders of magnitude within a larva. Such distribution is inconsistent with neural integrator models in which the activity of recurrently connected neurons approximates line attractor dynamics. Linear network model coefficients were calculated by direct fitting to experimental decay distributions. Simulated solutions to the model equations using fit coefficients recapitulate the decay distribution. Estimated connection strengths are excitatory and relatively strong for model neurons with similar decay times and weaker and often inhibitory for neurons with more disparate decay times.

GRASPING THE FLY'S COLOR VISION CIRCUIT

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The process of color vision requires the extraction of the wavelength information from the visual field by the comparison of at least two wavelength-sensitive sensors. We would like to understand how a neural wiring is able to support such process *in vivo*.

In Drosophila, the wavelength sensitive sensors are expressed within the inner photoreceptors called R7 and R8. They express the neurotransmitter histamine and project to the largest neuropil of the optic lobe, the medulla, which contains about 40 000 cells divided in at least 80 different cell types. Because no other neurons express histamine in the medulla, all the cells that express a histamine receptor are in theory directly downstream of at least one photoreceptor. Only one gene has been shown to encode a histamine receptor expressed in medulla neurons, *ort*, which is expressed in at least 5 different cell types in the medulla (1) .

The technique called GRASP, first developed in worm (2) and recently adapted to flies (3), allows the detection of synaptic connections between two populations of neurons, one under the control of a lexA driver and the other a gal4 driver. Using a recent library of Gal4 and LexA drivers expressed in the optic lobe, our aim is to determine how inner photoreceptors connect to the five different *ort*-positive cells. We are also investigating one step further in the connectivity, between these second order neurons and the next level of medulla neurons. Establishing the anatomical wiring of the medulla neurons is the first step in understanding the processing of color vision in the fly brain before testing their functional relevance.

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CONTRIBUTION OF GLUTAMATE AND GABA RECEPTORS TO SPATIOTEMPORAL DYNAMICS OF NEURONAL POPULATION ACTIVITY IN THE MOUSE VISUAL CORTEX

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Despite extensive knowledge of the basic blueprint of cortical circuits, detailed knowledge about functional connections and response dynamics of local cortical circuits is still limited. We addressed the questions of how NMDA and AMPA receptors differentially participated in spatiotemporal dynamics of neuronal population activity and how GABAa receptors shape excitatory transmission in local mouse V1 circuits, by using our recently developed technique which combines laser scanning photostimulation with voltage sensitive dye (VSD) imaging. Photostimulation via glutamate uncaging offered spatially restricted neuronal activation such that direct projections from the stimulated layer to its targeted layer(s) were detected by VSD imaging of evoked activation. Compared with cortical activity patterns in normal ACSF, we examined changes of spatial patterns and temporal dynamics of photostimulation-evoked V1 circuit activity under pharmacological blocking of NMDA or AMPA receptor activity. Application of the NMDA receptor antagonist, CPP (10 μM), clearly reduced translaminar and intralaminar propagation of photostimulation evoked responses in all laminar locations, with a more pronounced reduction occurring in layers 4, 5 than in layers 2/3. Relative to the peak activation size in normal ACSF, the reduction of average activation size in the presence of CPP was 64%, 76%, and 77.1% for layers 2/3, 4, 5, respectively (N = 3 slices). CPP also modulated temporal dynamics of response propagation by decreasing the initial propagation speed of evoked responses. In comparison, application of the AMPA/KA receptor antagonist, CNQX (10 μM), completely blocked translaminar signal propagation, and left residual responses in photostimulation sites. Thus, AMPA receptor activity plays a dominant role in determining the excitatory information flow in V1 circuits; whereas NMDA receptor activity can exert an important modulation on functional connections and spatiotemporal dynamics of local population activity. In the presence of GABAa receptor antagonist, bicuculline (2 μM), we found that early phase (< 35 ms post-photostimulation) of circuit responses was not significantly altered, and laminar projection patterns conserved, but the late phase (> 75 ms post-photostimulation) of excitatory responses was dramatically different as the average activation spread increased by 302%, 64%, and 320% from L2/3, L4, and L5 photostimulation, respectively (N = 4 slices). Taken together, our data demonstrate the requirement of both excitatory and inhibitory ionotropic transmission in maintaining and regulating functional connectivity and dynamics in visual cortex.

How Connectivity, Background Activity, and Synaptic Properties Shape the Cross-Correlation between Spike Trains

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Functional interactions between neurons in vivo are often quantified by cross-correlation functions (CCFs) between their spike trains. It is therefore essential to understand quantitatively how CCFs are shaped by different factors, such as connectivity, synaptic parameters, and background activity. We study the CCF between two neurons using analytical calculations and numerical simulations. We quantify the role of synaptic parameters, such as peak conductance, decay time, and reversal potential, and analyze how various patterns of connectivity influence CCF shapes. In particular, we find that the symmetry of the CCF distinguishes in general, but not always, the case of shared inputs between two neurons from the case in which they are directly synaptically connected. We systematically examine the influence of background synaptic inputs from the surrounding network that set the baseline firing statistics of the neurons and modulate their response properties. We find that variations in the background noise modify the amplitude of the cross-correlation function as strongly as variations of synaptic strength. In particular, we show that the postsynaptic neuron spiking regularity has a pronounced influence on CCF amplitude. This suggests an efficient and flexible mechanism for modulating functional interactions.

Physiological evidence for auditory cortex as a recursive parameter estimator

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Although animals are able to recognize a sound source in the presence of other sounds, the neural circuit that performs this function is unknown. We present an algorithm that performs a level-invariant recognition of the uncorrelated sources present in a mixture. A recursive version of this algorithm uses the estimated parameters to generate a prediction about sensory stimulus and uses the error in the prediction to adjust the estimated parameters. A circuit implementation of the recursive algorithm requires a large number of units in order to expand the prediction error of the input signal into errors of the estimated parameters and to represent the uncertainties associated to those estimates. We propose that the auditory cortex has the right input-output connectivity to implement this algorithm. Analysis of awake auditory cortical cells found groups of cells with characteristics that correspond to the predicted error signals and the associated uncertainties.

THE FUNCTIONAL ASYMMETRY OF AUDITORY CORTEX IS REFLECTED IN THE ORGANIZATION OF LOCAL CORTICAL CIRCUITS

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The primary auditory cortex is functionally organized tonotopically along the anterior-posterior cortical axis, whereas orthogonal to this axis neurons tend to respond to the same frequency (isofrequency axis). At the cellular level, whole-cell recordings *in vivo* have revealed how conductances shape auditory responses. However, there is a gap in our understanding of the link between stimulus representation and local circuitry. To make a direct connection, we combined *in vivo* recordings with functional circuit mapping *in vitro*. We targeted Layer 2/3 (L2/3) excitatory neurons in primary auditory cortex of juvenile mice using cell-attached recordings and juxtacellular filling with biocytin. We found marked differences in the responses of L2 and L3 cells: L2 showed strong tuning to pure tones of different frequencies and sweep direction, whereas L3 cells did not. To assess whether these differences in stimulus responses translate into functional circuitry differences, we used laser-scanning photostimulation (LSPS) to map presynaptic connections to L2/3 pyramidal cells *in vitro*. Slices were cut horizontally to preserve tonotopy and coronally to preserve isofrequency bands. Intralaminar connections provided the strongest input along both cortical axes. But surprisingly, we found that interlaminar connections along the tonotopic axis differed from those along the isofrequency axis. In the horizontal slice, L2 received columnar input from L5/L6 cells located directly beneath the patched cell, whereas L3 received off-center L5/L6 input (lateral distance 200-400 microns), biased mostly to the higher frequency side of the L3 cell. In the coronal slice, all input to L2/3 was columnar. L2/3 cells in auditory cortex also function more independently than in other cortical areas. The correlation of input maps between nearby cells (within 50 microns of one another) was roughly half of what was previously determined for neighboring barrel cortex neurons. Our results suggest that the microcircuitry of auditory cortex is specialized to the unique one-dimensional representation of sound in the auditory cortex.

PREEMPTIVE CONTROL OF BEHAVIOR BY A MECHANOSENSORY CIRCUIT IN *C. ELEGANS*

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Sensory circuits transform physical disturbances into neuronal signals providing sensory information to the nervous system. What is the impact of such sensory information on other circuits and how may it shape the way that they function and the behavior that they generate? We chose to address these questions using the well-described mechanosensory circuit of *C. elegans*, which senses gentle touch along the body of the worm. We compared various behavioral features between normal and touch-deficient worms in order to reveal the influence of mechanosensory information or lack of it on other neuronal circuits.

We found that touch-deficient worms behave differently from wild type. (1) When removed from their source of food touch-deficient worms restrict the range of their local search by increasing their reversal rate and turning size. (2) They suppress their head oscillations during all reversals, as opposed to wild type worms that suppress head oscillations only when reversing in response to anterior touch. (3) They show a decreased withdrawal response to aversive stimuli such as nose touch or the direct stimulation of the nociceptive neuron, ASH, using channelrhodpsin2. Calcium imaging experiments revealed corresponding differences in the activity of the command interneurons AVA/AVE that control backward locomotion and the RIM interneurons that control head oscillations and influence the frequency of reversals. These findings suggest that the absence of sensory information may lead to an enhanced restriction or a tighter control over behavior, preempting potential undesirable consequences of the worm's actions that could otherwise be avoided, if indeed they unfold, upon sensory detection.

How does the absence of sensory information induce such preemptive control? To address this question we examined worms with defects in genes that have been linked to plasticity and learning. We expected that worms that cannot sense touch but also lack functional plasticity molecules would exhibit wild type behavior since their circuits cannot learn to increase their preemptive control. Surprisingly, we found the opposite. Touch normal worms with a defective *hen-1* gene (encoding a neuronal secreted protein) phenocopied the enhanced preemptive control of touch insensitive worms in all of the behavioral features observed. Thus, a high degree of preemptive control may actually be the default condition and it is normal worms that learn to rely on the availability of sensory information to reduce the extent of preemptive control that they exert. We are currently tracking down the sites of activity of *hen-1* where we believe that the learning takes place, presumably through synaptic and/or intrinsic plasticity.

Zebrafish Behavioral Profiling Links Drugs to Biological Targets and Rest/Wake Regulation

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While exploring the mechanisms of sleep regulation in zebrafish, we developed and applied a high-throughput, quantitative screen for small molecule drugs that alter the behavior of zebrafish larvae. We found that the multidimensional nature of observed phenotypes enabled the hierarchical clustering of hundreds of molecules according to shared behaviors. Behavioral profiling not only revealed conserved functions of many psychotropic molecules that modulate the major neurotransmitter pathways but also predicted the mechanisms of action of poorly characterized compounds. In addition, behavioral profiling implicated unexpected factors such as ether-a-go-go-related gene (ERG) potassium channels and immunomodulators in the control of rest and locomotor activity. These results demonstrate the power of high-throughput behavioral profiling in zebrafish to discover and characterize psychotropic drugs and to dissect the pharmacology of complex behaviors. Our current work aims to identify the neuronal circuits modulated by small molecules and to link these neuronal populations to sleep/wake behavioral outputs. By linking molecular pathways to neuronal circuits to behavior, we hope to gain a more complete understanding of the regulatory mechanisms controlling sleep.

Increasing Adult Hippocampal Neurogenesis is Sufficient to Enhance Dentate Gyrus-dependent functions in Cognition but not Mood

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Adult hippocampal neurogenesis is a unique form of neural circuit plasticity that results in the generation of new neurons in the dentate gyrus (DG) throughout life. Adult-born neurons exhibit heightened synaptic plasticity during a specific temporal window of their maturation and can account for up to ten percent of the entire granule cell population. Although loss-of-function studies have informed our understanding of the role of new neurons in hippocampal functions, the impact of selectively increasing the number of new functional units in the DG on cognition and mood is not known. Here we show that inducible genetic expansion of the population of adult-born neurons in mice improves performance in a specific cognitive task in which an animal must distinguish between two similar contexts. Mice with increased functional integration of adult-born neurons show normal object recognition, spatial learning, rapid one trial contextual encoding, extinction learning and stable contextual fear memory but are more efficient in contextual fear discrimination learning, suggestive of enhanced pattern separation. Furthermore, mice with more adult-born neurons show greater exploratory behavior following voluntary exercise. In contrast, increasing adult hippocampal neurogenesis does not produce an anxiolytic or antidepressant-like behavioral response. Our findings suggest therefore that strategies designed to specifically stimulate adult hippocampal neurogenesis are likely to have pro-cognitive effects associated with improved pattern separation, but may not be sufficient to regulate mood. Furthermore, these studies begin to define the latent capacity of mature neuronal networks to integrate new functional units with concomitant improvement in discrete neural circuit functions.

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MOLECULAR ANALYSES OF THE INTEGRATION OF TWO SENSORY SIGNALS IN *C. ELEGANS*

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Environmental information received by sensory neurons is integrated in the central nervous system to regulate animal's behavior. The sensory integration can be considered as a simple processing for an adaptive response to environments. If the sensory information is improperly integrated, the resulting abnormal outputs will cause behavioral defects. Despite its importance, little is known about the mechanisms at the molecular level.

Genetic analyses by using *C. elegans* as a model system have advantageous features to examine the molecular mechanisms of the sensory integration. To elucidate the mechanisms, we carried out a genetic screen to identify genes required for the sensory integration between an attractant diacetyl and a repellent copper ion. In this screening, a copper ion barrier exists between diacetyl and worms and, thereby, worms must cross the copper ion barrier to reach the diacetyl spot. By this screening, we identified mutants deficient in *gcy-28*, a receptor-type guanylate cyclase, which less frequently crossed the barrier than wild type animals.

gcy-28 encodes four isoforms (*gcy-28.a-d*) by alternative splicing (Tsunozaki et al., 2008). GFP reporter analyses suggested that *gcy-28.d* isoform was expressed mainly in AIA interneurons. The expression of GCY-28.d by a promoter that gives the expression only in AIA interneurons was sufficient to restore completely the phenotype of the *gcy-28.d* mutant *tm3028* and partially rescued the defect of the mutant devoid of all *gcy-28* isoforms. These results suggest that *gcy-28* regulates the sensory integration in multiple neurons including AIA interneurons.

To investigate the genetic interaction between *gcy-28* and other genes involved in the sensory processing, we employed double mutant analyses in the sensory integration. Since GCY-28 is a guanylate cyclase, cyclic nucleotide gated channels could be candidates of the downstream component of GCY-28. Among those, *gcy-28;cng-1* double mutants showed the defect of the sensory integration as much as *cng-1* single mutants. On the other hand, *gcy-28;scd-2* double mutant showed stronger defects than each single mutant, although HEN-1/SCD-2 pathway also regulates the sensory integration in AIA interneurons. These results suggest that multiple pathways are involved even in a simple kind of sensory information processing, and provide insights into how sensory information processing is regulated in the nervous system.

A MAP OF *DROSOPHILA* BRAIN REGIONS AND FIBER BUNDLES

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We established a comprehensive brain map of *Drosophila melanogaster* by combining multiple techniques such as Bodian silver staining, pan-neuronal and pan-glial GAL4 driver strains, and various antibodies labeling specific molecules in the brain. By observing characteristic structures, as many as 40 neuropil areas with more than 100 substructures were described. Neuronal fiber bundles, or tracts, that connect multiple regions of the brain were also identified. A working group of researchers has been established since spring 2007 to discuss this issue, and the resulting proposal based on this work is going to be published. This map provides a new anatomical basis for *Drosophila* brain researches, with which characterization of higher-order neurons will be carried out smoothly.

We also developed an online database system of neurons in the fly brain, named Flybrain Neuron Database. Since the database is constructed intending to collect information of all identified neurons in the fly brain, information is mainly collected from published papers. Projection sites of the neurons were re-mapped according to the new terminology of the neuropils here we established. Currently 359 different types of neurons are inputted to the database, which may already cover the majority of identified types of neurons. The database also provides information on GAL4 and LexAV enhancer-trap strains as well as antibodies that label specific subsets of neurons.

Optical manipulation of fast-spiking interneurons in mouse barrel cortex during free behavior using channelrhodopsin-2

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In the mammalian neocortex, parvalbumin-positive (PV) interneurons provide fast somatic inhibition within a local network. By modulating spike probability in neighboring neurons with high temporal precision, these interneurons may exert powerful control over the "state" of a cortical region. There is strong correlative evidence supporting the importance of PV interneurons for generating high-frequency network activity, and selective activation of these cells has recently been shown to generate physiologically relevant oscillations in anesthetized animals.

To better understand the function of these cells in behaving mice, we combined optogenetic stimulation techniques with chronically implanted tetrode arrays. We introduced the light-sensitive ion channel, channelrhodopsin-2 (ChR2), into PV-Cre knock-in mice via transduction with a double-floxed virus. Several weeks after virus was injected into layers IV and II/III of somatosensory cortex, the ChR2 gene was expressed in PV cells over an area spanning multiple barrels. We used 473 nm laser light to activate PV interneurons while recording extracellularly near the stimulation site over a period of several weeks. To assess the relationship of driven activity in these neurons to active sensing in the mouse whisker system, we employed high-speed videography of whisking behaviors during spontaneous, novelty-driven exploration.

Our ability to manipulate a specific interneuron type motivated two general approaches. First, we used the response to a single pulse of light to probe ongoing network activity. We found that this evoked response is highly state-dependent, with differences in the ability to perturb the network implying differences in the ability to recruit PV interneurons or to induce their downstream effects. Second, we used optogenetics to trigger changes in neural and behavioral state. Single pulses of light could induce spindles from baseline or phase-reset ongoing spindle oscillations. Further, trains of light pulses at different frequencies could enforce rhythmic activity across a population of cells. In agreement with earlier findings, we found selective enhancement of gamma-range oscillations in the behaving animal. The emergence of these oscillations was highly state-dependent and correlated with behavior (e.g., whisking vs. non-whisking periods). In addition, transitions in sensory behaviors following epochs of light stimulation suggest the network perturbation is perceptually suprathreshold. Ongoing experiments will focus on predicting neural and behavioral responses as a function of the animal's current state and the parameters of the light stimulus.

Genetic dissection of cell types and neural circuits in the mouse brain

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In order to understand the enormous complexity of the brain it is essential to define its component parts, specifically its cell types and the connections formed between them. At the Allen Institute we are using a combination of approaches to determine the molecular, anatomical and functional properties of specific cell types in the mouse brain. We have identified candidate marker genes from the Allen Institute's *in situ* database, and combined *in situ* hybridization with anatomical tract tracing to determine the basic functional phenotypes of these molecular classes. Simultaneously, these genes have been used to create cre driver and responder transgenic mouse lines, as well as cre responsive viruses. These tools allow us to establish detailed circuitry and morphology for each putative cell type. Furthermore, by adding molecular tools to these genes, we are also able to image and manipulate the activity of these neurons *in vivo*. Using these viral and transgenic techniques we plan to establish neuronal circuits for a large number of specific cell types across the brain.

SOMATOSENSORY NEURAL CIRCUIT MAPPING IN LARVAL DROSOPHILA

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Not unlike humans, Drosophila respond to environmental stimuli including temperature, light, touch, smell, and taste. We are exploiting the relatively simple nervous system of the Drosophila larva to map mechano- and thermo-sensitive neuronal circuits. At approximately 10,000 neurons, the Drosophila larval nervous system is complex enough that the fundamental principles of neural circuit organization and function found in more complex nervous systems are present, yet simple enough that an in-depth understanding is realistic. The approach we are taking is to generate transgenic fly strains capable of expressing transgenes of choice in restricted subsets of neurons at all points of the circuit including the sensory, inter, and motor neurons. The regulatory regions used to generate these fly strains are derived from various trp sensory transduction channels, neurotransmitter synthesis enzymes, and neurotransmitter receptors. For sensory circuits of interest we are using optogenetic approaches to determine if particular neuronal subsets are necessary and/or sufficient for the behavioral output of the circuit, as well as GRASP to determine synaptic connectivity within the circuit. Our long-term goal is to understand the fundamental principles underlying sensory information processing.

Activity-dependent Regulation of Synaptic Transmission by the Metabolic Sensor, AMPK

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The generation of every action potential in the nervous system bears a substantial metabolic cost due to the large fluxes of ions required to depolarize the dendrites, soma, axon hillock and axonal arbor in turn. Consequently, neuronal metabolism and neuronal activity must be coordinated yet the mechanisms responsible for this regulation are poorly understood. Here, we examine the hypothesis that AMP-activated kinase (AMPK) serves such a role, responding to neuronal activity and regulating synapse function. We find that AMPK is basally active in neurons and bidirectionally regulated by neuronal activity. Furthermore, chronic but not acute disruption of AMPK catalytic activity perturbs spine morphology, suggesting that AMPK regulates synaptic development and morphology. Finally, a potential downstream mechanism in the regulation of spine morphology by AMPK is provided by the TSC2 protein, which is known to regulate protein translation and spine morphology, as TSC2 with mutated AMPK phosphorylation sites is unable to rescue the effects of TSC2 knock-down. Thus, AMPK, an evolutionary ancient metabolic enzyme, may play a specific, neuronal role in responding to and controlling synaptic activity.

Acid Sensing by the *Drosophila* Olfactory System

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The odor of acids has a distinct quality that is perceived as sharp, pungent and often irritating. How acidity is sensed and translated into an appropriate behavioral response is unknown. Determining whether acid sensation is mediated by a distinct group of neurons specifically tuned to protons (H^+) or by a complex pattern of activity across various cell types would be a first step toward unraveling the mechanism. Here, we describe a functionally segregated population of olfactory sensory neurons in the fruit fly, *Drosophila melanogaster*, that are highly selective for acidity. These OSNs express IR64a, a member of the recently identified Ionotropic Receptor (IR) olfactory receptor family. Until now, the function of IRs has not been addressed. Flies in which the function of IR64a+ neurons or the IR64a gene is disrupted have defects in acid-evoked physiological responses. But the responses to non-acidic odorants remained unaffected. IR64a is localized to the sensory dendrites, but not to the axonal processes, suggesting that IR64a constitutes a receptor that directly interacts with free protons. Together, these results reveal the identity of cellular and molecular substrates for acid detection in the *Drosophila* olfactory system and support a labeled-line mode of acidity coding at the periphery. These findings open up a possibility that other organisms, including humans, have an analogous olfactory mechanism to perceive acidity.

MEASUREING CODING PROPERTIES THROUGHOUT OLIVO-CEREBELLAR PATHWAYS WITH CALCIUM IMAGING

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The capacity to learn many motor responses and behaviors strongly depends upon normal function of olivo-cerebellar circuitry. One limitation to understanding how these circuits control or mediate learning has been the challenge of monitoring activity, as learning occurs, in the various cell types participating in the circuit. To overcome this difficulty and help identify potential sites of plasticity, we use two-photon calcium imaging with a larval zebrafish preparation to assess coding properties of olivo-cerebellar cells, including inferior olivary, granule, and Purkinje neurons. Cerebellar and olivary brain regions are loaded through bolus injection of acetoxymethyl- ester calcium dyes. After approximately one hour, cells show fast fluorescence changes of up to 80% that are correlated with spontaneous and optokinetic behavior. Loose-patch recordings from soma near the inferior olive show that such fluorescence changes are roughly linearly correlated with changes in firing rate. In the olive, cells do not show fluorescence changes related to saccades or fixations, but do show activity during optokinetic stimulus (1/32-1/8Hz) that peaks nearly in phase with the velocity of the eye in the ipsilateral direction (separate slip sensitivity has not yet been determined). A population of granule cells display strong correlation with ipsilateral saccades, and during optokinetic stimulation respond in phase with velocity in the contralateral direction. Purkinje cells in the vestibule-cerebellum display a variety of coding properties and directional preferences, with some showing strong optokinetic velocity sensitivity, others displaying mixed optokinetic position and velocity sensitivity, and others with strong eye position sensitivity. Significant spontaneous calcium signals and coordinated activation not related to oculomotor function were also observed throughout the olivo-cerebellar circuit. Together, these data support the idea that the vestibulo-cerebellum is capable of generating eye position signals to complement, or help train, brainstem circuits responsible for velocity-to-position integration. The approach enables characterization of coding properties, and changes in those properties, throughout the olivo-cerebellar circuit.

OLFACTORY NEURONAL CIRCUIT IN DROSOPHILA

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Light and electron microscopy both offer important advantages for characterizing neuronal circuitry in intact brains: LM can reveal connective patterns of the neurons between brain areas, and EM can confirm synaptic connections of identified neurons within a particular area. We sought to combine these techniques to characterize the Drosophila olfactory system.

To visualize olfactory tracts in Drosophila, we injected a Dextran-conjugated fluorescent marker into the antennal lobe (AL, the primary olfactory center). In addition to labeling fiber tracts identified previously, we also found several novel tracts leaving the AL and terminate in new targets. To analyze the projection patterns of individual neuron in these olfactory tracts, we also screened ~4,000 GAL4-enhancer trap strains and characterized about 200 that labeled olfactory neurons associated with the primary, secondary, and tertiary olfactory areas. Our approach revealed not only the projection patterns of olfactory neurons but also connections among primary to tertiary olfactory areas.

To analyze the synaptic connectivity in each olfactory area with EM, we then developed a dual-labeling method for pre-embedded brains. In Drosophila preparations with both marker injection and genetic labels, we visualized synaptic connections among two specific populations of neurons in the AL that have been shown to mediate odor evoked neural oscillations.

GENETIC DISSECTION OF GABAERGIC CIRCUITRY IN NEOCORTEX

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A prerequisite in understanding the organization, assembly and function of neocortical circuits is a systematic discovery and characterization of their diverse cell types. Despite a numerical minority in neocortex, GABAergic interneurons consist of a rich array of cell types with distinct morphology, connectivity, physiological properties, and gene expression profiles. These Inhibitory interneurons are crucial in establishing the balance, flexibility, and functional architecture of cortical circuitry, but the heterogeneity of cell types has been difficult to penetrate by conventional anatomical and physiological techniques. We use genetic strategies to dissect the GABAergic circuits by tapping into the intrinsic gene regulatory mechanisms that generate and maintain cell type diversity. We have generated over 20 knockin "driver lines" expressing Cre or the inducible CreER in all major classes of GABAergic neurons. Two broad categories of genes have been targeted: 1) transcription factors involved in the specification and differentiation of GABAergic neurons (*dlx1, dlx5, lhx6, nkx2.1*, and *er81*); "effector" genes expressed in all (e.g. *gad2*) or subsets of developing and/or mature GABAergic neurons (e.g. *parvalbumin, somatostatin, calretinin, cholecystokinin, vasoactive intestinal peptide, corticotropin releasing hormone, cortstatin*, and *nNOS*). We are characterizing these Cre drivers by crossing with several Cre-dependent GFP and RFP reporter lines. In sharp contrast to the transgenic approach, all knockin Cre lines appear to closely recapitulate endogenous gene expression pattern. In addition, an intersectional strategy combining overlapping expression of Cre and Flp allowed us to capture more specific subclasses. These GABA driver lines will facilitate progress in studying the development and function of GABAergic neurons and cortical circuits. We will present our latest progress on characterization of these drivers and discuss their applications with our ongoing experiments.

The structure of the afferent auditory pathway in Drosophila melanogaster.
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Auditory perception underlies several behaviors in Drosophila melanogaster, including courtship-related hearing. During courtship males flies generate a stereotyped wing-song towards their parters, but female flies and nearby male flies have distinct responses to this song. We have undertaken an enhancer-based screen to identify neurons underlying these responses. We outline the afferent auditory pathway for courtship song in D. melanogaster, and identify a wide variety of cell types within the antenno-mechanosensory and motor complex and related regions. In addition to a significant structurally diversity, the auditory circuit appears to be functionally sexually dimorphic: despite a common stimulus, several neurons are necessary for a behavioral response in only one sex. We discuss the implications of these findings for our understanding of both sensory processing and sexual dimorphism in the insect nervous system.

A Neurocognitive Graph Theoretical Approach to Understanding the Relationship Between Minds and Brains

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A fundamental goal of neuroscience is the development of quantitative models that explain the causal relationship between properties of the brain and properties of the mind. Here, graph theory is utilized to construct "brain-graphs", which are graphs with multi-edges and attributed vertices and edges. We prove that a kn nearest-neighbor (knn) classifier, under a Frobenius norm metric, is universally consistent, meaning that regardless of the (nonlinear) relationship between the brain and mental properties under investigation, given sufficient data, the knn classifier will achieve the optimal solution. We then simulate two populations of C. elegans: (1) wild-type, and (2) odor-evoked behavior impaired. Even in the relatively small sample limit (i.e., hundreds), the knn classifier achieves satisfactory performance. Current technology could enable confirming this result in actu. Because the knn classifier is model free, we are currently developing model-based classifiers, with the intent to reduce classifier variance without introducing too much bias.

HOW DOES A WORM MOVE?

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An animal's locomotory behaviors arise from a complex interplay between neurons, muscles, and the external environment. However, a detailed mechanistic understanding of locomotory behavior, which traverses the inner workings of an animal's sensory and neuromuscular networks and the mechanical interactions of the animal with its surroundings, does not yet exist. Here, we study the neural and biomechanical basis of locomotion in the roundworm *Caenorhabditis elegans*, a small model organism that moves by generating sinusoidal dorsal and ventral body bending waves. We focus on two questions with a combined experimental and theoretical approach: (1) How does the motor circuit generate and propagate sinusoidal undulations? (2) What is the role of mechanosensory feedback in specifying the undulatory wave?

On the experimental side, we observed that *C. elegans* continuously adapts its gait from swimming to crawling when we gradually increased the mechanical load on a swimming animal by changing the fluid viscosity. By analyzing spatial transitions of a worm between fluids with different viscosities, we found that during forward locomotion, the undulation frequency is likely controlled by the motor circuit near the head while the wavelength of undulation is affected by the local mechanical load along the body. To probe the role of mechanosensory feedback in more detail, we used microfluidic channels to control spatiotemporally the body curvature of a swimming worm. We found that during forward locomotion, body wall muscles receive stretch sensory feedback from the neuromuscular networks in the anterior segments.

On the theoretical side, we postulate the following phenomenological model of worm forward locomotion. The motor circuit near the head has two stable states, which lead to dorsal or ventral body bend. Stretch sensory feedback, which is induced by body curvature, triggers the transition between the two states. The oscillation propagates from head to tail through stretch sensory feedback that couples local body segments. The relation between muscle contraction and body curvature depends on the biomechanics of muscles and local mechanical environment. Our model accounts for the above experimental observations and the predictions of the theory will be discussed to inspire future experiments.

Molecular neuroimaging of dopamine with a novel MRI contrast agent

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Dopamine (DA) is a key neurotransmitter in the brain that has received special attention because of its role both in normal brain function and disease. Traditionally, the DA network has been partitioned into a *nigrostriatal pathway* (connecting the substantia nigra (SN) with the dorsal striatum), involved in motor control and perturbed in Parkinson's, and a *mesolimbic pathway* (connecting VTA and ventral striatum), involved in reward processing and pathologically altered in addiction.
However, this proposed dichotomy has recently been challenged by anatomical and behavioral findings that show overlapping projection fields of the two dopaminergic cell populations as well as involvement of both systems in reinforcement and addictive behavior.
In order to functionally dissect the DA network, fine-grained detection of the spatiotemporal patterns of DA transmission is thus critical. However, this can not be accomplished with conventional point measurements like voltammetry or with low resolution imaging using PET.
We have therefore developed the first DA-specific molecular imaging agent that detects DA dynamics quickly, reversibly and specifically over large regions of the brain using high resolution non-invasive MRI.
The novel dopamine sensor is based on the metalloprotein P450-BM3 (BM3h), a genetically encodable MRI contrast agent whose sensitivity and specificity for dopamine was greatly improved using directed evolution techniques. Binding of dopamine to a site close to the protein's paramagnetic heme iron reduces the T1 relaxation rate and leads to a relative MRI signal decrease.
In vivo experiments show that coinjection of the sensor with exogenous dopamine into rodent brain results in a clearly observable hypointense MRI signal, compared to areas injected with sensor alone. In addition, potassium challenge of the rat forebrain leads to a significant MRI signal decrease mediated by the sensor. Ongoing experiments in the lab seek to apply the sensor for imaging phasic dopamine release during electrical stimulation of the *mesolimbic pathway* to generate spatiotemporal maps of DA transmission as a function of differently rewarding stimulation.

GENETIC ANALYSIS OF BEHAVIORAL PLASTICITY AND LEARNING IN ZEBRAFISH

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In response to experiences with their environment, animals constantly update their behavior through the processes of sensorimotor integration and learning. The simplest form of learning is non-associative learning, which is defined as a change in attention directed towards a stimulus. For example, abrupt sensory stimuli evoke an evolutionary conserved motor response, the startle reflex. The startle reflex is stereotyped but highly modifiable, such that repeated presentation of startling stimuli suppresses the startle response. This decrease in attention towards an irrelevant stimulus is defined as habituation and represents plasticity at the level of the circuit driving the suppressed behavior.

Zebrafish show a remarkable capacity for behavioral plasticity, and using an automated high-speed video analysis and tracking system, we show that zebrafish larvae (5-14 days post fertilization) exhibit robust short-term habituation of the acoustic startle response with landmark behavioral and pharmacological characteristics. Importantly, our assay identifies habituation of a very specific sensorimotor behavior - only performed in response to startling stimuli - controlled by a well characterized and simple neural circuit that is easily visualized in an intact, freely behaving vertebrate organism. Therefore, we can pinpoint the site of synaptic plasticity to a specific component of the circuit and identify the locus of genes regulating this plasticity.

Here, we show that our how habituation assay satisfies the nine behavioral characteristics commonly used to define habituation behavior3. For example, we show that more frequent stimulation results in a more rapid and pronounced response decrement with more rapid spontaneous recovery and that habituation can be reversed through cross-modal stimulation (dishabituation). These results argue in favor of plasticity driving the habituation and against fatigue as an explanation for the decreased responsiveness to repeatedly presented acoustic stimuli. Finally, we describe the first zebrafish learning mutants, identified from a Tol2-mediated gene breaking insertional mutagenesis screen. These mutants exhibit a significant reduction in startle short-term habituation, but are neither hypersensitive to the acoustic stimulus nor are they hyperactive. We are currently investigating the ability of these mutants to recall long-term habituation to acoustic stimuli and are working towards cloning the locus of the gene breaking insertion to identify the affected gene.

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Visualizing the activation and plasticity of GABAergic neurons through activity-regulated transcription of the Gad67 gene

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In mammalian brains, GABA-mediated synaptic transmission regulates the balance, flexibility, and dynamics of neural circuit operations. In addition, GABA signaling regulates many aspects of neural development, from cell migration to synapse formation and plasticity. Although there is a large diversity of GABAergic neurons that release GABA and a similarly large diversity of GABAA receptors that signal GABA, there is only mechanism to synthesize GABA – by conversion from glutamate through a single decarboxylation step. Among the two GABA synthetic enzymes, GAD67 is the rate-limiting enzyme, and alterations in GAD67 levels readily influence the cellular and vesicular GABA contents. The major step at which GAD67 activity is controlled physiologically is gene transcription, which is dynamically regulated during development, and by neural activity and experience. Alterations of Gad67 transcription likely influence not only inhibitory transmission but also inhibitory synapse development and plasticity. Importantly, there is evidence that Gad67 is differentially regulated in different interneuron cell types in various physiological and pathological states (e.g. significant reduction in parvalbumin- and somatostain- interneurons in the prefrontal cortex of schizophrenic patients). However, currently there is no method to monitor Gad67 transcription with cell type resolution in vivo.

We have generated a knock-in mouse line, Gad67-d2EGFP, which express a destabilized (2h half life) form of EGFP under the control of the endogenous Gad67 promoter. We show that d2GFP fluorescence reliably reported transcriptional regulation of Gad67 bi-directionally in response to pathological (limbic system seizure), pharmacological , physiological (visual deprivation, whisker stimulation and deprivation) stimulus. We find that GAD67-d2GFP is differentially regulated in distinct classes of interneurons with different time courses after sensory experience. In summary, the Gad67-d2GFP reporter allows visualization of the activity and likely plasticity of GABAergic neurons with cellular and cell type resolution.

Describing the different classes of local interneurons and their role in olfactory information processing in Drosophila antennal lobe

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Drosophila antennal lobe (AL) is becoming more and more popular for studying the olfactory information processing. Relatively small number of neurons and ease of genetic manipulations provide us a unique system to understand neural circuit function when combined with electrophysiology. In the level of projection neurons (PNs) olfactory information processing is well investigated. Recent studies showed that local interneurons (LNs) play important roles in neural computations within the AL. However little is known about types and functions of various classes of LNs.

We performed intracellular recordings in different GAL-4 lines expressing fluorescent proteins in subsets of LNs and showed that there are distinct classes of LNs with various morphologies and electrophysiological properties and thus may be playing different roles in olfactory information processing in Drosophila AL.

Next, we performed intracellular dual patch recordings (LN- LN or LN-PN pairs) in order to understand the functional connectivity within the fly antennal lobe and roles of different classes of LNs in odor information processing. Our preliminary results demonstrated that most of the LNs receive direct; strong and global excitatory input from most of the PNs. We observed both inhibitory and excitatory effects of LNs on PNs or other LNs. Our findings set a ground work for a more detailed investigation of lateral inhibition and lateral excitation and their functional roles.

A large scale screening of GAL4 lines to search for command neurons in the *Drosophila* brain

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Understanding the synaptic mechanisms that act during development to construct and organize behavioral circuits is a central challenge in neuroscience. To meet this challenge it will be necessary to identify networks that control distinct behaviors and to study their development and interactions. Command neurons, which when stimulated trigger a defined motor pattern(1), are particularly attractive candidates for these types of studies. In *Drosophila*, only the giant fiber neurons which control the escape response, have been definitively identified as command neurons. To identify novel command neurons, we have used genetic tools for targeted neuronal activation to screen a large set of enhancer-trap Gal4 lines.

As a resource for seeking command neuron circuits, we took advantage of GAL4 lines generated by the NP project(2). We crossed one thousand GAL4 strains with relatively limited GAL4 expression in the brain to flies carrying TRPM8, a cold-sensitive ion channel. Low temperatures opened the TRPM8 channel in GAL4 expressing cells leading to their excitation and consequently induced a variety of behaviors in adult flies. 83 lines exhibited paralysis, likely due to the generalized disruption of motor processing or the activation of inhibitory pathways, while 47 lines showed specific behaviors. Many of the elicited behaviors were simple, such as jumping or wing-raising, although the latter could be part of a complex behavior such as seen in male to male aggression. In some cases, activation of GAL4-positive cells triggered more elaborate motor programs, such as feeding behavior, egg laying in females, or the entire courtship ritual in males, indicating that a complex behavioral sequence could be triggered by activation of small numbers of neurons. These potential command neuron circuits could be used to study CNS synaptic plasticity related to behavior.

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ENDOCANNABINOID-DEPENDENT LTD REQUIRES TRPV ACTIVATION.

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Endocannabinoids are neurotransmitters known to mediate both short- and long-term forms of synaptic depression. Our laboratory has shown that leech (*Hirudo verbana*) central synapses are capable of undergoing an endocannabinoid-dependent form of long-term depression (ecLTD) following 900sec low frequency (1Hz) stimulation (LFS). The presence of endocannabinoid transmitters in invertebrates is well-established, but the receptors that these transmitters bind to is unknown given that orthologues of mammalian CB1 and CB2 receptors are absent in protosomal invertebrates. However, endocannabinoids have shown to bind to TRPV channels and mediate some forms of ecLTD in the mammalian CNS. It is possible that these channels mediate ecLTD in invertebrate synapses that lack the classic CB1 and CB2 receptors.
In the leech, both nociceptive (N-cells) and non-nociceptive touch cells (T-cells) have synaptic input onto the same longitudinal (L) motor neurons. LFS of the T-to-L pathway elicits heterosynaptic LTD (hetLTD) in the N-to-L synapse. Application of RHC-80267, which inhibits the synthesis of the endocannabinoid transmitter 2-AG, blocked the hetLTD in the N-to-L synapse. This hetLTD was also blocked by capsazepine, an inhibitor of TRPV receptors, indicating that TRPV activation was required for hetLTD. Application of capsaicin, an activator of TRPV receptors, or the endocannabinoid transmitter 2-AG, mimicked LFS-induced LTD and both capsaicin and 2-AG-induced depression were blocked by capsazepine. Finally, 2AG and capsaicin occluded subsequent LFS-induced LTD.
The significance of these findings is two-fold. First, it suggests that ecLTD, as a result of TRPV activation, is a conserved form of synaptic plasticity found in both vertebrates and invertebrates. Second, the ability of LFS of the T-to-L pathway to elicit hetLTD in the leech N-to-L synapse in may provide a useful model system for investigating how activation of non-nociceptive pathways alters synaptic transmission in nociceptive neurons.

A Mouse Retinal Ganglion Cell that Detects Object Motion

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The mouse retina has at least 20 different types of retinal ganglion cells (RGCs), which deliver visual information to the brain. Each type of RGC tiles the retina such that their combined receptive fields fully cover the visual field. Thus each RGC type conveys a complete but uniquely processed visual image to the brain. To analyze these representations, we are generating transgenic mouse lines in which different types of RGCs are selectively labeled by YFP. We then characterize the response properties of the RGCs using targeted cell attached and whole cell recordings. Here we describe one such RGC type termed W3-RGCs. W3-RGCs are bistratified ON-OFF cells with several interesting characteristics: First, this is one of the most numerous RGC types in the mouse retina. They have very small dendritic fields, and correspondingly small receptive field centers. Second, the inhibitory surround of the receptive field is disproportionally large and also unusually strong. For example, a light spot centered on the receptive field but slightly larger than the center completely fails to elicit action potentials from the W3-RGCs. Third, the W3 cell shows non-linear spatial summation in both the center and the surround areas, and for both the ON and the OFF pathways. This is a characteristic of ganglion cells that signal pattern motion in the image. Interestingly, we found that W3-cells respond to motion in the receptive field center regardless of the direction of motion, but only if the image in the surround moves with a different trajectory. Thus they are poised to report the motion of an object relative to its background, even if the entire retinal image jitters due to head and eye movements. We further probed the synaptic mechanism underlying this sensitivity. It appears that W3 cells receive transient inhibition elicited by motion in the receptive field surround. This precisely timed inhibition acts both presynaptically and postsynaptically to suppress simultaneous excitatory input from the receptive field center. Thus the W3 cell's response is suppressed if the motion trajectory in the center is the same as in the surround. Given their high density and small receptive fields, the W3 cells likely determine the acuity limit of mouse vision. Yet, unlike in the primate retina, these cells report the result of sophisticated visual computations.

Expression of beta amyloid induced age-dependent presynaptic and axonal changes in Drosophila

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Alzheimer's disease (AD) is due to synapse dysfunction and loss, but the nature and progression of the presynaptic structural and functional changes in AD are largely unknown. We expressed wild-type or arctic form of beta amyloid1-42 (Aβ) in a small group of neurons in the adult fly and performed extensive time-course analysis of the function and structure of both axon and presynaptic terminals at the identified single-neuron level. Aβ accumulated intracellularly and induced a range of age-dependent changes, including depletion of presynaptic mitochondria, slowdown of bi-directional transports of axonal mitochondria, decreased synaptic vesicles, increased large vacuoles, and elevated synaptic fatigue. These structural and functional synaptic changes correlated with age-dependent deficit in motor behavior. All these alterations were accelerated in flies expressing arctic form of Aβ. The depletion of presynaptic mitochondria was the earliest detected phenotype, and was not caused by the change in axonal transport of mitochondria. Moreover, axonal mitochondria exhibited a dramatic reduction in number but a significant increase in size in aged Aβ-expressing flies, indicating a global depletion of mitochondria in the neuron and an impairment of mitochondria fission. These results suggest that Aβ accumulation depletes presynaptic and axonal mitochondria, leading to other presynaptic deficits.

Retrograde and trans-synaptic tagging of neurons with pseudorabies virus amplicons

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Most brain regions are composed of heterogeneous mixtures of neurons with diverse patterns of inputs and outputs. Neurons with different patterns of connectivity may exhibit different physiological properties and play different roles in the circuit. To explore the functional roles of these different neuronal classes, we are developing tools which allow us to target GFP, channelrhodopsin-2 (ChR2), or other genes to specific neuronal classes on the basis of their connectivity.
Pseudorabies virus (PRV) is a neurotropic alpha-herpesvirus, which has been used extensively for polysynaptic circuit tracing in rodents. However, PRV is relatively toxic to neurons, which limits its utility in long-term physiological studies. Furthermore, because of its large genome (~150 kb), specialized molecular techniques are required to engineer the virus to express new genes such as ChR2.
To overcome these limitations, we have optimized PRV amplicons for functional analysis of neural circuits. These amplicons are pieces of DNA which carry all the necessary signals required for packaging into PRV virions, but carry none of the PRV genes responsible for toxicity. PRV amplicons retain the ability of PRV to enter into axons terminals and undergo retrograde transport to the soma, and under the appropriate conditions can also undergo trans-synaptic transport. PRV amplicons offer several advantages over alternative retrograde viruses such as rabies or herpes simplex virus. First, as noted above the absence of viral genes in the amplicons minimizes toxicity. Second, the carrying capacity of PRV amplicons is large, potentially as high as about 150 kb. Third, manipulation of PRV amplicons is straightforward, amenable to conventional restriction cloning. Finally, because each PRV virion packages about 150 kb, a typical 10 kb amplicon will be present in multiple (i.e. 15) copies, resulting in strong expression of the transgene.
In the presence of helper PRV, amplicons can move trans-synaptically between connected neurons in both retrograde and anterograde directions. The PRV glycoprotein B (gB) is essential for trans-synaptic spread: PRV virions lacking gB are incapable of infecting or spreading from neuron to neuron. However, gB can be supplied in trans, so that a neuron expressing gB can support the transport of PRV amplicons, along with the gB null PRV helper, to a synaptically coupled partner. If this partner neuron does not express gB, then neither the amplicon nor the helper virus is propagated further. In this way, the spread of amplicons can be restricted to monosynaptically coupled partners. Amplicons can be used to directly drive expression of genes of interest such as ChR2 or GFP. Alternatively, amplicons expressing cre can be used to turn on expression of genes from cre-dependent viruses. These viral tools could be combined with optogenetic techniques to selectively monitor and manipulate the activity of anatomically defined populations of neurons.

Dynamic Brain States

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All areas of the cortex continually undergo experience-dependent functional changes throughout life. These changes underlie a form of memory known as perceptual learning as well as functional recovery following CNS damage. Cortical plasticity is mediated by rapid and massive rewiring of cortical circuits. On an even more rapid time scale the function of each cortical area is determined by powerful top-down influences including attention, expectation and perceptual task. As a consequence every cortical area acts as an adaptive processor, undergoing continuing cycles of state change and functional switching. During perceptual learning, both the encoding and recall of learned information involves a selection of the connections that are appropriate for the perceptual task being performed.

Synaptic Selectivity of Neocortical Circuits

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An essential step in understanding cortical function is determining the patterns of selective connections among the different neuronal types in neocortical circuits and the properties of their synapses. This step requires robust definitions of cell type to investigate connections among identified classes of neurons. A number of approaches have been used to target different cortical cell types and probe the properties of their connections. These studies suggest that patterns of connections in local neocortical circuits and their synaptic properties depend on cell type, both for inhibitory GABAergic neurons and excitatory pyramidal neurons.
Simultaneous intracellular recordings from inhibitory neurons have demonstrated a remarkable specificity of chemical and electrical synaptic connections. For example, parvalbumin-expressing fast-spiking (PV-FS) cells form GABAergic connections onto neighboring PV-FS cells while somatostatin-expressing Martinotti cells do not synapse onto each other. Furthermore, electrical synapses are common among PV-FS cells and among Martinotti cells but rarely do these two cell types form electrical synapses with other cell types. Recently, we showed that the probability of connection among pyramidal neurons depends on both the presynaptic and postsynaptic cell types. By comparing the physiological probabilities of connection of pyramids with different long-range projections to their local axo-dendritic overlap, we showed that the specific rates of connectivity among L5 pyramids cannot be solely explained by their anatomical relationships, indicating that other factors allow pyramidal cells of different functional classes to selectively connect.
Progress in understanding the patterns of connectivity among cortical neurons and the properties of their synapses has been greatly facilitated by the development of new approaches for identifying cortical cell types. For example, nearly all major types of inhibitory neuron can be targeted for physiological study using transgenic mice expressing fluorescent proteins under the control of cell-type specific promoters. This approach has rapidly shown additional specificity of chemical and electrical synapses among GABAergic neurons. Using a similar genetic approach, light-gated channels can now be introduced in particular cell types to probe the connectivity of cortical neurons. The properties of unitary connections can then be compared using paired recordings. The combination of these genetic, anatomical and physiological approaches promises to reveal a detailed picture of the functional circuitry of local neocortical circuits.

High Precision and Fast Functional Mapping of Cortical Circuitry Through A Novel Combination Of Voltage Sensitive Dye Imaging And Laser Scanning Photostimulation

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The development of modern neuroscience tools is critical for deciphering brain circuit organization and function. We have developed a new functional mapping technique in brain slices which incorporates the spatial precision of activation that can be achieved by laser-scanning photostimulation with rapid and high-temporal resolution assessment of evoked network activity that can be achieved by fast voltage-sensitive dye (VSD) imaging. Unlike combination of whole cell recordings with photostimulation for mapping local circuit inputs to individually recorded neurons, this innovation is a new photostimulation-based technique to map functional circuit output and connections at the level of neuronal populations. Here we report on this technique, and demonstrate its effective applications in mapping functional connections and circuit dynamics in mouse primary visual cortex (V1) and hippocampus.

We first demonstrate high precision and rapid mapping of V1 circuits through VSD imaging and photostimulation at multiple V1 laminar locations. Photostimulation in V1 cortical layers initiated excitation which resulted in VSD signals first localized to the stimulation site at around 10-20 ms after laser exposure; excitation then propagated to functionally connected cortical regions. For layer 2/3 stimulation, the activation in layer 2/3 was mostly localized in layer 2/3, however, relatively small but clear activation propagated from layer 2/3 to layer 5, bypassing most of layer 4. Stimulation in layer 4 caused strong excitatory activity to spread vertically to layers 2/3 and 5. Layer 5 stimulation resulted in distinct foci of activation in layer 2/3, and some activation spread into layers 6 and 4. Thus, the new technique enables direct visualization of interlaminar functional connectivity in mouse V1 at a previously unattainable precision and speed. We further extended our technique to investigation of the hippocampal circuitry. We probed different hippocampal locations with spatially restricted photostimulation and imaged the initiation and propagation of evoked responses throughout the hippocampal circuitry. With the new technique, we have perhaps for the first time demonstrated that activation of a small subset of hippocampal dentate gyrus (DG) neurons clearly engaged serial excitatory propagation in DG, CA3 and CA1. In addition, we examined the effects of GABAergic inhibition on hippocampal circuit connections and dynamics, by comparing neuronal population excitability, spatial patterns and temporal dynamics of evoked activity in hippocampal slice preparations in normal conditions and after blocking GABA receptor-mediated inhibition. Taken together, our new technique can provide important applications in cortical circuitry studies.

Coordinated Regulation of Inhibitory and Excitatory Local Circuits by Experience During Development of Layer 4 Barrel Cortex

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A critical step in the development of primary sensory cortex is maturation of layer 4, which receives a large proportion of ascending thalamic input to neocortex and contains a precise map of the sensory periphery. In rodent barrel cortex, this map is of the facial vibrissae and during the first postnatal week the map is highly sensitive to whisker-driven sensory experience. Neocortical circuits require a close interplay of inhibition and excitation that together produce a transformation of input into appropriate output. In the case of layer 4, the thalamocortical projection is an important input and the excitatory projection neurons, stellate cells, provide the output. We have thus investigated developmental and experience-dependent changes in local inhibitory and excitatory circuits in layer 4 that are driven by thalamic input and assessed effects on stellate cell output.

We analyzed inhibitory and excitatory circuits using simultaneous patch-clamp recordings and 2-photon imaging/uncaging of inhibitory interneurons and stellate cells in a thalamocortical slice preparation from mice aged postnatal day 4 – 12, a period when layer 4 is sensitive to sensory experience. We found that feed forward inhibition, driven by thalamic input onto stellate cells, emerges over approximately 2 days at postnatal day (P)8-9 and involves a number of changes in the circuit. Feed forward inhibition when it emerged serves to strongly down regulate stellate cell spiking in response to thalamic input and set dendritic integration. The recruitment of feed forward inhibition requires sensory experience that selectively strengthens the thalamic input onto fast spiking interneurons; other developmental changes in the inhibitory circuit occur independent of whisker experience. Experiments are now currently underway to assess the role of experience in development of feed back inhibition.

To investigate development of the recurrent excitatory network in layer 4, we developed a technique using 2-photon glutamate uncaging to map stellate cell to stellate cell connections. In the first postnatal week connectivity was very low (~4 %), but increases threefold over one day at P9, a time that coincides closely with the emergence of feed forward inhibition. Analysis of structural changes in the same cells shows there is a spinogenesis in stellate cells at P9, in which cells progress from having no spines to hundreds of spines in a period of 1 day. Experience drives the threefold increase in functional connectivity, but is not required for the spinogenesis. Current work using 2-photon glutamate uncaging on spines is investigating mechanisms for experience-dependent regulation of spine function.

Taken together our analysis of layer 4 circuit development reveals highly coordinated, rapid, experience-dependent changes in inhibitory and excitatory circuits to produce a layer 4 network that can transform input into appropriate output.

Molecular genetic dissection of the whisker barrelette circuit

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We are interested in understanding the formation of barrelette structures and mapping the micro-structures within each barrelette.

The rodent whisker map is a preeminent example of a continuous somatosensory map. Yet we discovered that sensory neurons innervating neighboring whiskers within the same row show no spatial organizations inside the trigeminal ganglion, rather their cell bodies appear randomly distributed along the anterior-posterior (A-P) axis of the ganglia. To restore order in their central axonal projections, trigeminal neurons with dis-continuous positions rely on whisker-derived TGFβ signaling to form the precise whisker map. Deleting Smad4, a common transducer of TGFβ signals, exclusively in sensory neurons, disrupts normal brainstem barrelette patterns representing individual whiskers within the rows. The defects are caused by axon mis-projections and abnormal segregations. Our results reveal a previously un-recognized discrete component of the somatosensory map and highlight the crucial role of peripheral target-derived signals in conveying the topographic information in the somatosensory system.

Each whisker is innervated by several different types of mechanosensory neurons (including Merkel, longitudinal lanceolate, transverse lanceolate, Ruffini/spiny, and reticular endings), thus each barrelette is an integration site of different types of tactile inputs. We asked whether there are fine scale organizations within the barrelette such that distinct types of sensory afferents project to distinct domains within each barrelette? We analyzed mice in which Merkel endings axons are specifically labeled. We found that Merkel ending neurons send collaterals to all four brainstem nuclei. 3D reconstructions of Merkel projections within a single barrelette will be presented.

Neuronal biophysics modulate the ability of gamma oscillations to control response timing.

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Gamma frequency activity of inhibitory sub-networks has been hypothesized to regulate information processing in the cortex. Inhibitory neurons in these sub-networks synchronize their firing and selectively innervate the perisomatic compartments of their targets, generating both tonic and rapidly fluctuating inhibition that is hypothesized to enforce temporal precision and coordinate the activity of their post-synaptic targets. Indeed, in vivo and in vitro recordings have demonstrated that many neurons' firing is entrained to these oscillations, although to varying extents and at various phases. Cortical networks are composed of diverse cell populations that differ in chemical content, biophysical characteristics, laminar location, and connectivity. Thus, different neuronal types may vary in the amplitude and timing of the synchronized inhibition they receive, as well as in their response timing and precision. What accounts for this heterogeneity of response timing between cell types, and are these responses fixed or flexible?
To answer these questions, we use a combination of in vitro electrophysiology, dynamic clamp, and modeling to characterize the interactions between a neuron's intrinsic properties, the degree of gamma-band synchrony among its inhibitory inputs, and its spike timing. We apply these techniques to study six distinct types of cortical neurons. We find that neuron types systematically vary in the phase and precision of their spike timing relative to the peak of gamma frequency input, and the degree to which their spike time depended on changes in inhibitory synchrony. Biophysical characterizations of real neurons suggest that the membrane time constant (Tm), afterhyperpolarization amplitude and duration, and sodium channel properties are key features governing gamma control of response timing. We tested these findings both in a single compartment model, and by using dynamic clamp to alter intrinsic features of neurons' physiology.
We conclude that a neuron's intrinsic physiology substantially affects the ability of gamma-synchronized inhibitory inputs to control its response timing. These results suggest that the characteristic phase relationship of neuronal discharges during gamma activity may be explained by differences in intrinsic properties as well as bydifferences in connectivity. Further, we note that the relevant physiology is not static, but may be altered by contextual or neuromodulatory factors. We therefore suggest that the ability of gamma oscillations to control response timing is not fixed, but may be dynamically shaped to suit the cortex's computational requirements during attention or cognition.

Differential sensitivity of different sensory cortical areas tobehaviorally relevant millisecond-scale differences in neural activity

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Animals can detect the fine timing of some stimuli. For example, in subcortical structures, submillisecond interaural time differences are computed to determine the spatial localization of sound. Although cortical neurons have not been shown to achieve comparable submillisecond precision, neurons in auditory, visual and barrel cortex can lock with millisecond precision to the fine timing of some stimuli. However, the ability of these cortical neurons to fire precisely does not demonstrate such fine timing is behaviorally relevant.

To bridge the gap between physiology and behavior, we have previously used electrical microstimulation to determine the temporal precision with which fine differences in cortical spike timing could be used to drive decisions. We found that in rat auditory cortex, animals could be trained to use timing differences as short as 3 msec to drive decisions (Yang et al, Nat Neurosci 11, 1262-3).

Is the auditory cortex unique in its ability to utilize such fine timing differences to drive behavior? Because audition is often considered to be a "fast" modality---one in which subtle differences in temporal structure can be behaviorally relevant---it would not be unreasonable to speculate that the auditory cortex had evolved special mechanisms for rapid processing. On the other hand, it is appealing to hypothesize that the cortex operates according to general principles shared across different regions; in this view, the ability to make use of millisecond-scale differences in neuronal activity would not be unique to the auditory cortex.

To distinguish these hypotheses, we compared the ability of different sensory areas to resolve subtle differences in neural timing. In the visual cortex, we found that although animals could be trained to resolve differences as short as 15 msec in neuronal activity, they could not resolve differences as short as 5 msec. This lower limit of 5-15 msec was significantly higher than the limit of 3 msec we had observed in auditory cortex, and is consistent with the view that visual cortex is "slower" than auditory cortex. Surprisingly, we found that the barrel cortex was even "faster" than auditory cortex, with a lower limit below 1 msec. Our results suggest that different cortical areas are differentially able to derive behaviorally relevant information from the fine timing of neural activity.

Critical Features of Network Connectivity

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I will discuss features of neuronal circuit connectivity required to support spontaneous activity and stimulus-evoked responses with complex dynamics, as well as the transfer of information between neurons. These features include sufficiently strong excitatory synapses with balancing or dominant inhibition, a hierarchy of network interactions with an appropriate level of sparseness, and a high degree of variability between network elements. Experimental signatures of these features will be addressed.

NOVEL CLASSES OF OPTICAL NEURAL CONTROL TOOLS REVEALED VIA SCREENING OF PHYLOGENETIC DIVERSITY

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The heterologous expression of light-activated proteins to enable optical physiological perturbation of genetically specificed cells, a field often termed “optogenetics”, is a powerful methodology in neural circuit analysis, allowing for spatio-temporal assessment of the necessity and sufficiency of neural activity patterns in brain functions. By screening ecological diversity, we have discovered several stimulation and silencing tools for controlling neural activity over timescales relevant to the probing of neural computation and behavior (Halo/NpHR, Han and Boyden, *PLoS One* 2007; ChR2, Boyden et al. *Nature Neuro* 2005). We here expand upon these tools by exploring entire classes of efficacious neural silencers that build upon our earlier characterization of the novel molecules Arch and Mac (Chow and Han et al. *Nature* 2010), which enable extremely powerful silencing, and blue-light silencing, respectively.

First, we report several new neural silencers from the *Halorubrum* family, related to Arch, that have up to four-fold improved light-sensitivity, enough to enable significant regions of the non-human primate brain to be cleanly, reversibly, and safely silenced. Furthermore, we report on early approaches for enhancing multicolor silencing, including variants of fungal and archaeal opsin that enable well-separated blue vs. red light driving. Finally, we report a fourth class of optogenetic microbial opsins that can mediate both neural activation and silencing in response to two different colors of light, opening up the possibility of precise dialing-in of neural conductance using the full color spectrum range. In summary, boundaries between classes of opsin provide fundamental principles for the identification and design of opsins of desired kinetics, color, and sensitivity, based largely on their phylogenetic origin. We are using these opsins to discover principles of optical neural control that will enable researchers to optimally apply given tools from specific classes toward neuroscientific discovery and the therapeutic treatment of abnormal pathologies.

Towards a Genetic Dissection of Neocortical Circuits: Targeting GABAergic Interneurons

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Although the neocortex consists of distinct functional areas, cortical networks arise from duplication of stereotyped circuit modules, with subtle specializations across different areas and mammalian species. Superimposed upon the cellular complexity in cortical circuitry, there is stunning stereotypy in cellular components and their connectivity, which also manifest across areas and species. A thorough characterization of these cellular components is necessary for understanding the assembly and function of cortical circuits. Although a minority, GABAergic interneurons regulate the balance, dynamics, and functional architecture of cortical circuitry. Diverse GABAergic cell types display distinct physiology, connectivity patterns, and gene expression profiles, but pose a major challenge for experimental and theoretical studies. Using mouse genetic engineering, we have systematically established reliable "genetic access" to major classes of GABA neurons; we generated ~20 knockin driver lines expressing Cre or inducible CreER in defined classes of inhibitory interneurons.

A useful criterion for classifying cortical GABAergic neurons is the cellular and subcellular specificity of their axon targeting, such as: 1) distal dendrite-Martinotti cells, 2) soma and proximal dendrite – PV and CCK basket cells, 3) axon initial segment – chandelier cells, 4) non-synaptic volume transmission - neurogliaform cells, 5) interneuron selective – bipolar etc. Characterization of GABA Cre drivers indicates that our genetic strategy captured most of these anatomically and physiological defined cell classes. In addition, an intersectional strategy combining Cre and Flp lines further increased specificity, suggesting that highly distinct cell types can be captured by overlapping two driver systems. These genetic tools allow reliable and systematic visualization and functional manipulation of defined GABAergic cell types, and also enable cell type-based genomic analysis of molecular profiles. A number of driver lines allow us to visualize the "life history" of subsets of GABA neurons (e.g. dendrite-targeting somatostatin interneuron), from their birth in ganglionic eminence to their action in mature cortical circuits. We are examining whether and how cell lineage and developmental history influence the assembly of GABA neurons into cortical circuits. Our current progress will be presented.

Simultaneous Multi-electrode Recording and Anatomical Identification of Retinal Ganglion Cells

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Understanding the function of a neural circuit requires knowing the diverse cell types that compose it. Cells types are commonly defined using either anatomical or physiological criteria, but few methods exist to link the two. For example, in the primate retina anatomical surveys have described the morphology of nearly every type of retinal ganglion cell, including their respective brain projections. At the same time, new multi-electrode recording methods can characterize light responses of hundreds of ganglion cells simultaneously, defining new functional cell types as well as painting a rich physiological portrait of each type. Here we describe a method to match anatomical and physiological classes of primate retinal ganglion cells using an in vitro preparation. The match is based on associating the morphological features of labeled cells with the electrical footprint of extracellularly recorded spikes.

Macaque RGCs were labeled with EGFP expressed via a modified rabies virus injected into the LGN that infected axon terminals (Wickersham et al, 2007). EGFP expression was strong and apparently labeled the full extent of individual RGCs of several types, producing clear images of dendritic arbors and axons. ON and OFF midget and parasol cells, the four numerically dominant RGC types in primate retina, were identified by their distinctive morphology. Retinas with labeled neurons were recorded using high density multi-electrode arrays. In these recordings, midget and parasol cells were identified based on their light response properties and density. The challenge was then to uniquely match a labeled cell to its recording.

The coarse morphology of a recorded cell was summarized by its electrophysiological image (EI; Litke et al 2004): the average voltage waveform on all electrodes around the time of a spike. Each EI was then compared to each labeled cell. The recorded cell with the EI most closely matching a given anatomical image, in terms of soma location, dendritic field, and axon trajectory, was identified as the imaged cell's match. The match was independently validated using information about cell type and mosaic organization. Matched cells were found to have the same morphological and functional cell type, and occupy approximately the same location in the mosaic.

This technique provides a robust link between large-scale electrical recording methods and the underlying anatomical organization of neural circuits. In the future, this technique can be extended to other cell types in the retina, or other systems, for which the correspondence between anatomical and functional cell types is not known.

Automated high-throughput microscopy for fluorescent mouse brain

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We have developed high-throughput fluorescence microscopy for whole mouse brain, which integrates high-speed two-photon imaging and automated tissue sectioning. The instrument works as follows: 1) fixed brain is placed under the microscope on a computer-controlled motorized stage, which moves the sample in x-y direction; 2) once the top view is mosaic-imaged, the brain is moved towards a built-in vibratome to cut the imaged section; 3) the brain is moved back and the cycles of imaging and sectioning are repeated. Currently, the time to image an entire mouse brain at a resolution matching the Reference Allen Brain Atlas (140 sections at x-y resolution of 1.2 μm) is 6 hours. This technology thus allows automated, rapid imaging of mouse brains at cellular resolution. We demonstrate the use of this method for mapping both anatomy and function of mouse brain circuits. Anatomical connectivity of neural circuits is mapped by whole-brain imaging of traditional fluorescent tracers and recombinant viruses expressing GFP. Functional mapping of neural circuits is done by whole brain imaging of immediate early gene (IEG) induction in transgenic "indicator" mice, which express GFP from IEG promoters, such as c-fos-GFP and Arc-GFP mice.

SPATIOTEMPORAL ODOR PRESENTATION IN MICROFLUIDIC ARENAS TO INTERROGATE *C. ELEGANS* NEURAL CIRCUITRY

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Goal-directed animal behaviors represent the output of information processing by neural circuits. As a model toward understanding circuit computation, we study odor chemotaxis behavior of the nematode *C. elegans*. The worm navigates via a biased random walk, in which turning frequency is modulated by sensory neural activity. However, mapping information flow along this circuit is hampered by poor control over sensory input in typical agar-plate assays. To address this limitation, we developed a series of microfluidic arenas that enable the quantitative study of subtle locomotory responses to precise spatiotemporal odor patterns. Stimulus gradients can span several cm or change sharply (<50 μm) across an animal, and remain highly stable or change rapidly within <1 sec. Multiplexed devices track up to 100 animals and 4 genotypes for hours. Moreover, these devices can be prepared inexpensively from plastic molds, enabling their use in standard lab settings.

We recorded instantaneous behavioral states in wild-type *C. elegans* subjected to temporal odor pulses (10 s to 5 min). Stochastic responses varied across animals, but population-wide state probabilities changed robustly within seconds of each stimulus change, subject to adaptation across multiple time scales. Next, we measured behavioral responses to spatial stripe and ramp odor patterns to understand how animals modulate speed and turning events in varied environments. Similar studies of genetic mutants with disrupted neuronal activity revealed patterns of information flow that direct these behavioral motifs. We also observed that nearly all (99%) wild-type animals showed head-directed steering up sharp spatial gradients (~10 μM/mm), inconsistent with a random walk and suggesting neural circuitry capable of computing the direction of a stimulus gradient. Overall, the quantitative and systematic analysis of output responses to precise stimuli, coupled with remote readouts and control of neural activity, will represent a powerful toolset for dissecting and modeling the neural basis of behavior.

Representations of odor in the piriform cortex

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Olfactory perception is initiated by the recognition of odorants by a large repertoire of receptors in the sensory epithelium. A dispersed pattern of neural activity in the nose is converted into a segregated map in the olfactory bulb. How is this representation transformed at the next processing center for olfactory information, the piriform cortex? Optical imaging of odorant responses in the cortex reveals that the piriform discards spatial segregation as well as chemotopy and returns to a highly distributed organization in which different odorants activate unique but dispersed ensembles of cortical neurons. Neurons in piriform cortex, responsive to a given odorant, are not only distributed without apparent spatial preference but exhibit discontinuous receptive fields. These results from imaging experiments are in accord with anatomic tracing that indicate that projections from a single glomerulus are not spatially segregated but broadly innervate the piriform cortex. This representation suggests organizational principles that differ from those in neocortical sensory areas where cells responsive to similar stimulus features are clustered and response properties vary smoothly across the cortex

Organization of olfactory bulb projection to the cortex revealed by retrograde mono-transsynaptic labeling

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In the mouse olfactory system, each of the >1000 classes of the olfactory receptor neurons sends convergent axonal projections into a pair of specific glomeruli in the olfactory bulb (OB), creating a space map for odor detection. Projection neurons in the OB, the mitral/tufted cells, receive ORN inputs from individual glomeruli and send their axons to the olfactory cortex. How the olfactory map in the OB is represented in the cortex is largely unknown. Here we combine a recently developed retrograde mono-transsynaptic labeling technique (Wickersham et al., Neuron 53, 639, 2007) with mouse genetics for in vivo tracing of synaptic connections. We restrict spatial location of "starter" populations to a few neurons of a specific type, and visualize their presynaptic targets with single-cell resolution. Using this method, we systematically mapped mitral cell connections to different olfactory cortical structures. We found single cortical cells in olfactory cortices on average receive input from mitral cells representing at least 3-14 different glomeruli, demonstrating a direct convergence of information represented in different glomerular units in individual cortical cells. Small areas of the olfactory cortex can receive broad array of glomerular inputs. However, quantitative examination reveals different degrees of topographic organization in the anterior olfactory nucleus, amygdala and piriform cortex with respect to their glomerular input. These results indicate that the space map in the OB is differentially represented in different parts of the olfactory cortex. This genetically regulable transsynaptic labeling method to trace projection at a single neuron resolution in vivo can be widely applied to map connections in many parts of the nervous system in mice.

Light addressable glomeruli reveal non-redundant odor coding of sister mitral cells in the mouse

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Sensory inputs frequently converge in a spatially organized manner to the brain. This organization typically leads to overlapping response properties in the target neurons, and it is important for sensory information processing to understand whether the output coding of these neurons is redundant or independent. To address this question in the olfactory system, we generated channelrhodopsin-2 expressing transgenic mice in all olfactory sensory neurons and selectively stimulated individual glomeruli using a digital micro-mirror device to achieve patterned optical stimulation. We investigated the functional properties of mitral/tufted (M/T) cells that receive inputs from the same glomerulus (sister cells). Here we show that sister M/T cells have highly correlated responses to odors as measured by average spike rates, but spike timings are significantly different when measured as a function of respiration phase. In contrast, non-sister M/T cells correlate poorly on both these measures. We suggest that sister M/T cells carry two independent channels of information: average activity representing shared glomerular input, and phase-specific information that refines odor representations and is substantially independent for sister M/T cells. It is of great interest to determine if and how downstream brain structures decode this phase-specific information.

Cross-talk between olfactory processing channels

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The Drosophila antennal lobe is a useful model circuit for investigating olfactory processing. The compartmental organization of the antennal lobe makes it relatively easy to map connections between neurons, and each compartment (or "glomerulus") corresponds intuitively to a discrete processing channel in the network. Moreover, this circuit contains relatively small numbers of neurons, and genetic tools allow us to label identified cells for recording. We monitor in vivo odor responses in these neurons using electrophysiological recordings, and we use genetic tools and pharmacology to probe their functional interactions. I will describe recent experiments from our laboratory investigating the mechanisms and functions of connections between glomeruli.

COMPREHENSIVE MAP OF THE *DROSOPHILA* PRIMARY GUSTATORY CENTER REVEALED WITH TWO ENHANCER TRAP SYSTEMS

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A main taste sensory organ of a fly lies on the tip of its mouth called labellum. Unlike mammals, gustatory receptor neurons (GRNs) in the labellum send their axons directly to the brain through the labial nerve. This feature, as well as the simple brain structure and wide availability of genetic tools, makes *Drosophila melanogaster* a suitable model for tracing neural circuits of the gustatory system. Through a large-scale screening of 4,000 GAL4 enhancer-trap strains, we obtained five lines that visualized specific subsets of the GRNs as well as one line that selectively labeled putative taste-associated mechanosensory neurons. We also found a LexA::VP16 (LexAV) enhancer-trap strain that labeled landmark structures in the primary gustatory center (PGC). By double labeling with these GAL4 and LexAV strains, we identified the precise positions of the axon terminals of various GRN subtypes and established a projection map in the PGC consisting of eleven zones, among which five were novel. Projection targets of known GRNs were also mapped in this framework. This comprehensive sensory map revealed remarkable projection patterns of GRNs: 1) segregated projections of the putative mechanosensory neurons and GRNs, suggesting separated processing of the chemo- and mechanosensory signals associated with gustatory sensation, and 2) converged projection of multiple types of GRNs, suggesting possible integration of different taste signals in the PGC.

A GROUND PLAN UNDERLYING THE ORGANIZATION OF MOTOR NETWORKS IN HINDBRAIN AND SPINAL CORD

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All of the motor output from the nervous system comes from the hindbrain and spinal cord. These regions contain many different specialized networks that control movements of the eyes, gills, jaws, diaphragm, head, limbs, and body. Our recent work has focused on the question of whether there are principles of organization that are shared by motor output networks throughout the neuraxis. We have found striking parallels between the patterns of development and functional organization of networks involved in the control of zebrafish movements. The different cell types in the networks are generated by a transcription factor code, which gives rise to core sets of neurons sharing axonal projection patterns and transmitter phenotype. Components are drawn from these sets to build particular motor circuits. Neurons arising from within individual transcription factor domains vary systematically in their properties based up their time of differentiation. Early differentiating neurons have lower input resistances, larger axonal fields, and higher thresholds for activation. Younger neurons have more restricted projections and are increasingly more excitable the younger they are. A motor circuit such as that underlying swimming, which operates over a broad range of speeds or strengths, draws neurons from a range of ages. These neurons are then recruited during behavior according to age, with the youngest ones recruited at the lowest speeds and increasingly older ones activated as the behavior increases in speed and strength. A behavior involving extremely fast movements over a narrow speed range, the escape bend or C-start, draws components selectively from the oldest cells in different transcription factor domains. This organization is most obvious in our recent studies of the hindbrain of young (not embryonic!) swimming larval zebrafish, which have all of the primary motor behaviors evident in the adult, and indeed, must execute them well in order to stay alive. The hindbrain neurons in these animals are arranged into a series of transcription factor/transmitter stripes. Neurons within stripes share gross morphological features and are arranged along the axis of the stripes by age, and, in turn, morphological and functional properties. Thus, an orderly ground plan is present at a time when the core networks are wired and functional, suggesting that the networks are built according to simple rules at a time when the brain is well patterned. Later migration and intermingling of cells obscures the structural and functional patterning that may lie at the foundation of motor networks throughout the brain and spinal cord.

Genetic Specification of Swimming or Walking Behavior by Motor Neurons

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It is known that in the spinal cord of primitive fish such as the lamprey, motor neurons are arranged in bilateral motor columns while in mammals, the bilateral medial motor column (MMC) is largely replaced by the lateral motor column (LMC) that is found at the lumbar level to supply the muscles of the limb. Behaviorally, the locomotor central pattern generator (CPG) organization in fish produces (in addition to right-left alternation) a smooth, propagating-wave phase relationship along the body axis. In mammals, the CPG also produces right-left alternation with striking flexor-extensor alternation at the lumbar levels of the cord to control limb movements with opposing muscles. Here we show that ectopic expression of the LIM homeodomain transcription factor, Lhx3, in motor neurons not only respecifies all motor neurons to a MMC identity, it also respecifies the behavior of the flexor-extensor phase of the mouse CPG to a phylogenetically more ancient phase like that of swimming animals, while preserving period, rhythmicity, and left-right alternation. Because the behavioral organization of the cord is altered electrophysiologically, we predicted circuitries would be altered in a detectable manner. Using principle component analysis of eletrophysiologic signals we detected circuitry changes within spinal cord segments that are dependent on these discreet changes in motor neuron identity. This provides a genetically parsimonious mechanism to generate diverse neural circuitries throughout the central nervous system by genetically specifying a minimum of diverse cells within a plastic circuit.

Integrating parallel actions onto a small motor circuit

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Studies using bath-applied and neuronally-released modulators in the crustacean stomatogastric system have helped establish that neuronal circuits are multi-functional. Recently, we have extended these studies by using insights from computational modeling to perform dynamic clamp manipulations of an intrinsic current to characterize the influence of sensory feedback, hormonal modulation, and the parallel influence of both pathways on a well-defined, neuronally-modulated motor circuit.

These studies focus on the two-phase (protraction, retraction) gastric mill (chewing) rhythm in the stomatogastric ganglion (STG) of the crab Cancer borealis, driven by tonic stimulation of the multi-transmitter projection neuron MCN1. In brief, the core gastric mill circuit includes the reciprocally inhibitory protractor neuron LG and retractor neuron Int1, plus the STG axon terminals of MCN1 (MCN1STG). MCN1 drives the gastric mill rhythm by slow, peptidergic excitation of LG and fast, GABAergic excitation of Int1. Both events occur during retraction, because LG inhibits MCN1STG during protraction. MCN1 excites LG by activation of the modulator-activated inward current (IMI-MCN1). The retractor phase (~5 sec) persists until sufficient IMI-MCN1 builds up in LG to enable it to overcome inhibition from Int1 and fire a burst. The protractor phase (~5 sec) persists until the LG burst self-terminates, due to the decay of IMI-MCN1 caused by LG inhibition of MCN1STG.

Sensory Feedback: The proprioceptor GPR slows the MCN1-elicited gastric mill rhythm (MCN1-GMR) by selectively prolonging retraction, due to its slow presynaptic inhibition of MCN1STG during this phase of the rhythm. GPR also excites Int1 and inhibits LG, but neither synapse is functional during this rhythm. Further, despite having 3 cotransmitters, GPR uses only 5HT to inhibit MCN1STG. This synaptic action selectively weakens MCN1 peptidergic excitation of LG, having no effect on the MCN1 GABAergic excitation of Int1. This selective regulation of peptide transmitter release is necessary for the normal GPR regulation of the MCN1-GMR.

Hormonal Modulation: The peptide hormone CCAP slows the MCN1-GMR by modestly prolonging protraction. Although CCAP excites several gastric mill neurons, including Int1, this CCAP action on the MCN1-GMR results from its convergent activation of IMI (IMI-CCAP), with MCN1, in LG. While CCAP does not alter retraction duration, its presence during retraction is necessary to maintain the control duration of this phase. Surprisingly, the IMI-MCN1 and IMI-CCAP dynamics in LG are distinct, due to only IMI-MCN1 being regulated by LG inhibition. Dynamic clamp manipulations of IMI-CCAP in LG establish that its influence in LG, including its differential dynamics relative to IMI-MCN1, is necessary and sufficient for CCAP modulation of the MCN1-GMR.

Hormonal Modulation of Sensory Feedback: Surprisingly, despite not altering retraction duration itself, CCAP weakens or eliminates the GPR prolongation of the retractor phase. Computational modeling and dynamic clamp manipulations of IMI-CCAP show that this action results from this current compensating for the slower buildup of IMI-MCN1 in LG during retraction when GPR is inhibiting MCN1STG. These results show that modulation of sensory feedback not only occurs at the level of the sensory neuron and/or its postsynaptic target, but can result from downstream actions on the target circuit.

Collectively, these studies of identified modulatory inputs to a modulator-driven motor circuit indicate that the effects of parallel modulation on neuronal circuit activity can result from novel cellular and synaptic mechanisms that provide these circuits with unexpected additional degrees of freedom. SUPPORT: NS42813, NS29436.

Innexins Coordinate Neuronal Communication of the *C. elegans* Motor Circuit

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Locomotory circuits integrate various sensory inputs, then determine and execute appropriate locomotory responses. We use the *C. elegans* nervous system as a model to address cellular and molecular mechanisms that underlie the function and modulation of the motor circuit.

Wild-type *C. elegans* move both forward and backward in smooth sinusoidal waves. Our behavioral analysis of a class of *C. elegans* mutants called kinkers suggest that their locomotion defects may be caused by uncoordinated neuronal activity in the motor circuit: they fail to propagate sinusoidal waves that drive forward locomotion; and they are completely biased toward backward movement.

Previous anatomical reconstruction of the *C. elegans* nervous system (White *et al*., 1984) and single neuron ablations (Chalfie *et al*., 1985) have proposed two separate motor circuits that control locomotion: forward movement is governed by the AVB and PVC interneurons activating B-class excitatory motoneurons; whereas AVA, AVD, and AVE interneurons activate the A-class motoneurons to drive backward movement. We established methods to monitor interneuron and motoneuron activity using the calcium sensor cameleon and applied them to 1) verify the proposed locomotory circuits in wild-type animals; and 2) examine the defects in the locomotory circuits of kinker mutants.

We will present unpublished results that suggest 1) instead of two independent circuits, a coordination between A and B motoneuron activity is required for directional locomotion in wild-type animals; and 2) such coordination is disrupted in kinker mutants, likely due to the hyperactivation of the circuitry that favors backward locomotion.

At the molecular level, the loss of function mutations in two innexin-encoding genes, *unc-7* and *unc-9*, lead to kinker phenotypes. While the UNC-7 functions mainly in backwards-driving interneurons, UNC-9 functions mainly through motoneurons. Taken together, we propose that *C. elegans* locomotion depends on the coordinated activity of the two motor circuits. Such coordination requires innexins, the invertebrate pannexin channels, to inhibit the motor circuit that drives backward locomotion.

THE WORMS CRAWL IN, THE WORMS CRAWL OUT – BEHAVIORAL AND OPTOGENETIC ANALYSIS OF THE *C. ELEGANS* LOCOMOTORY CIRCUIT

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The nematode *C. elegans* moves forward by propagating sinusoidal dorso-ventral bending waves from head to tail, a behavior generated by a circuit of command neurons, motor neurons, and muscles. While functional roles have been ascribed to different circuit elements via laser ablation experiments, relatively little is known about how the circuit generates and propagates undulatory waves. Here we describe our analysis of the motor circuit using quantitative behavioral assays of worms in structured environments and during ChR2/Halo-mediated perturbations. We first examined how the motor circuit is capable of supporting two locomotory modes, swimming and crawling, which differ in frequency and wavelength. By quantifying worm locomotion in viscous fluids, we showed that locomotory patterns continuously depend on mechanical load. We then examined worm locomotion during transitions between regions of high and low viscosity, and found that undulatory frequency and wavelength depend both locally and globally on the worm's mechanical loading. Next we investigated the behavioral effects of optogenetic perturbation of muscles and motor neurons, using a novel optical illumination system capable of arbitrary spatiotemporal perturbation of neural circuit activity in freely behaving worms. Analysis of the recovery from transient optically-induced paralysis of body wall muscles suggested that the locomotory pattern is encoded in posture-independent internal states. Local optical inhibition of muscles at mid-body caused the amplitude of bending waves to be reduced in not only the illuminated region but also posterior to the illuminated region during forward movement. Similarly, immobilization of the worm at mid-body using microfluidic channels caused immobilization of the posterior region during forward movement, suggesting that feedback from body curvature is required for wave propagation. We discuss implications of our results for theoretical models of the *C. elegans* motor circuit.

A stochastic model of command neuron function in *C. elegans*

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Locomotory state in *C. elegans* is regulated by a network of forward and reverse command neurons in two reciprocally connected pools, but how this network functions is poorly understood. We propose a model in which the network acts as a stochastic system. The model is based on three assumptions: (i) Forward neurons act as a single unit F and reverse neurons act as a single unit R. (ii) Unit activation switches stochastically between two activation states: 0 and 1, corresponding to off and on, respectively. (iii) The rate of a 0-1 transition, written a01, is a monotonic increasing function of synaptic input, i.e. the weighted sum of presynaptic activation states.

Given two neurons and two activation states, there are four possible states of the network (F,R) = {(0,0), (0,1), (1,0), (1,1)}. Previous neuronal ablations suggest that the first three states correspond, respectively, to locomotory pauses, forward locomotion, and reverse locomotion; we propose that the state (1,1) also corresponds to pauses.

The kinetics of the network are fully described by eight rate constants. Using a maximum likelihood procedure, these quantities can be estimated from empirically determined probability density functions for three behavioral states (Forward, Reverse, and Pause) together with the time series of velocities recorded for individual worms.

Synaptic interactions in the model are described by six coefficients: the two cross connections (W_{FR}, W_{RF}), the two self-connections (W_{FF}, W_{RR}), and the two connections representing sensory input (h_R, h_F). Assuming that the forward and reverse motor systems can be approximated as semi-linear Hopfield neurons, the synaptic coefficients can be estimated from the rate constants. The resulting model recapitulates a wide range of *C. elegans* behaviors. For example, both the touch-induced escape response and the gradual transition from local search to ranging behavior can be modeled by making h_R and h_F functions of time, whereas chemotaxis can be modeled by making the h_R and h_F functions of the local chemical gradient. We conclude that the stochastic switch model provides an intuitive yet predictive representation of command neuron function.

Crosstalk in plasticity and signaling between nearby synapses on hippocampal dendrites

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Long-term potentiation (LTP) of synaptic transmission underlies aspects of learning and memory. LTP can be input-specific, even at the level of individual synapses, suggesting that single synapses can function as independent units of memory storage. Synapse-specificity could be achieved through compartmentalization of synaptically-triggered signal transduction events in dendritic spines. To examine the functional isolation of individual synapses, we studied the interactions between plasticity at neighboring spines in hippocampal pyramidal neurons in brain slices. Repeated activation of NMDA receptors on individual dendritic spines, by two-photon glutamate uncaging or synaptic stimulation, induced synapse-specific LTP and a persistent spine enlargement. LTP and spine enlargement were restricted to the spine receiving the LTP stimulus. Following LTP induction, subthreshold stimuli, which by themselves were too weak to induce LTP, now caused robust LTP and spine enlargement at neighboring spines. This reduction in the threshold for LTP induction lasted ~10 minutes and spread over ~10 μm of dendrite. Furthermore, we used two-photon fluorescence lifetime imaging to measure the spatial activity dynamics of the small GTPase Ras during the induction of LTP at individual spines. Ras activity spread by diffusion from the potentiated spine and invaded neighboring spines over ~10 μm of dendritic length, indicating that spines do not compartmentalize all Ca^{2+}-dependent signaling events. The spread of Ras-dependent signaling was necessary for the reduction in LTP induction threshold at nearby spines. These local interactions between neighboring spines suggest that synapses are not functionally isolated units and support clustered plasticity models of memory storage. Clustered plasticity could allow for the binding of behaviorally-linked information on the same dendritic branch.

I will also present my recent work from David Tank's laboratory on the subthreshold membrane potential dynamics underlying rate and temporal coding in hippocampal place cells in mice navigating through a virtual reality environment.

NETWORK MECHANISMS OF THETA RELATED NEURONAL ACTIVITY IN HIPPOCAMPAL CA1 PYRAMIDAL NEURONS

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Oscillatory activity is a ubiquitous feature of nervous systems. A conspicuous 4-10 Hz theta oscillation can be observed in many regions of the mammalian nervous system in both extra- and intracellular recordings. Theta oscillations are particularly prominent in the hippocampus during exploratory activity and place cell firing rate and timing are characteristically related to theta phase. Although this represents one of the best examples of temporal coding in the mammalian brain, little is known about the specific biophysical mechanisms involved. Recent intracellular recordings have revealed additional features of place cell activity that implicate a particular abstract oscillatory interference model of hippocampal theta activity; the soma-dendrite interference (SDI) model. Here we attempt to gain insight into the underlying cellular and circuit level mechanisms of theta activity by implementing a form of somato-dendritic interference using the actual hippocampal network. Using whole-cell recordings from CA1 pyramidal neurons together with photo-stimulation of ChR2-expressing GABAergic interneurons, we have examined the ability of divisive perisomatic inhibition to produce the characteristic features of theta related activity. We found that pairing increasing levels of phasic dendritic excitation with phasic stimulation of perisomatic projecting inhibitory interneurons induced a somatic polarization and action potential timing profile that closely mimicked most but not all common features. Alterations in the temporal profile of inhibition, consistent with endocannabinoid-induced modulation of presynaptic GABA release properties, were required to fully capture all features. Other implementations largely fail to produce the appropriate results. Together these data support the concept that theta-related place cell activity is generated by an interaction between a phasic dendritic excitation and a phasic perisomatic shunting inhibition delivered by interneurons a subset of which experience activity-dependent modulation of their presynaptic properties. The utilization of these particular biophysical mechanisms could provide the neuronal network with functional advantages associated with a high degree of excitatory and inhibitory input compartmentalization.

Neuronal Population-Level Memory Traces in the Mouse Hippocampus

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What are real-time memory patterns in the brain? Can we decode neural populations' memory traces as the subjects form and recall memories? How meaningfully can those memory traces in the brain be related to a behavioral readout? Here, we describe real-time neural ensemble transient dynamics in the mouse hippocampal CA1 region and demonstrate their relationships with behavioral performances during both learning and recall. We employed the classic trace fear conditioning paradigm involving a neutral tone followed by a mild foot-shock 20 seconds later. Our large-scale recording and decoding methods revealed that conditioned tone responses and tone-shock association patterns were not present in CA1 during the first pairing, but emerged quickly after multiple pairings. These encoding patterns showed increased immediate-replay, correlating tightly with increased immediate-freezing during learning. Moreover, during contextual recall, these patterns reappeared in tandem six-to-fourteen times per minute, again correlating tightly with behavioral recall. Upon traced tone recall, while various fear memories were retrieved, the shock traces exhibited a unique recall-peak around the 20-second trace interval, further signifying the memory of time for the expected shock. Therefore, our study has revealed various real-time associative memory traces during learning and recall in CA1, and demonstrates that real-time memory traces can be decoded on a moment-to-moment basis over any single trial.

CHOLINERGIC INTERNEURONS MEDIATE FAST GLUTAMATERGIC TRANSMISSION IN THE STRIATUM

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Interneurons, which do not project axons beyond their local area, play a critical role in determining the activity of their neighbors in structures throughout the brain. Because their somata are interspersed among their cellular targets, experimental challenges to selectively stimulating these cells have limited analysis of their synaptic connections. Within the striatum, cholinergic interneurons (CINs) are the sole source of acetylcholine, a neuromodulator that is necessary for normal motor function and habit learning and that is perturbed in a number of neurological disorders including Parkinson's disease. While the actions of acetylcholine on striatal function are well-studied, surprisingly little is known about the synaptic properties of these neurons. Here, we took advantage of optogenetic tools to selectively activate CINs in acute brain slices of the mouse striatum. We used a recombinant adeno-associated virus approach to transgenically express the blue light-activated cation channel Channelrhodopsin-2 (ChR2) under control of the choline-acetyltransferase promoter. Brief light pulses (473 nm) were capable of reliably driving CIN spikes. Simultaneous recordings from medium spiny neurons, the principal projection cells in the striatum, revealed fast inward currents time-locked to the light stimuli. These currents produced depolarizing postsynaptic potentials in current-clamp recordings and were resistant to blockade of both GABA and acetylcholine receptors. Instead, they were sensitive to antagonism of AMPA- and NMDA-type glutamate receptors. Histological analyses confirmed that CINs are positive for the type-3 vesicular glutamate transporter. These results indicate that CINs are capable of releasing glutamate via an action potential-dependent mechanism. As CINs are tonically active *in vivo*, our findings suggest that, in addition to providing cholinergic modulation, these interneurons may also provide a background tone of glutamate receptor activation important for the regulation of striatal activity.

Optogenetic investigation of the synaptic circuitry of neostriatal cholinergic interneurons

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Cholinergic interneurons are one of the main neuromodulatory systems controlling the activity of the neostriatum. Like nigrostriatal dopaminergic neurons cholinergic interneurons exhibit brief firing responses which are precisely timed to the presentation of behaviorally significant stimuli and encode critical information about the incentive and attentional significance of these external events. An important problem is understood how these brief signals are detected and decoded by the striatal circuitry. The behaviorally relevant population response of cholinergic interneurons consists of a brief period of cessation of firing termed the pause response which in most cases is followed and sometimes preceded by a similarly short-duration increase of firing rate. Because of the partially inhibitory nature of this response and because of the spatial distribution of cholinergic interneurons in the striatal network classical methods cannot be used to reproduce these firing patterns or study their effects on the striatal network. Here we expressed the inhibitory light-driven chloride pump Halorhodopsin selectively in cholinergic interneurons in order to reproduce these population responses under experimental control. Since these neurons are spontaneously active in vitro, population responses consisting of a pause and pause-excitation sequences similar to those observed in behaving animals can be reproduced using optical inhibition alone. Recordings from striatal projection neurons and other GABAergic interneurons demonstrate that, in addition to other effects the pause and pause-excitation responses directly regulate the activity of projection neurons by controlling the activity of at least 2 distinct population of GABAergic interneurons through nicotinic mechanisms. These findings reveal the existence of a previously unrecognized complex circuitry of cholinergic and GABAergic interneurons in the striatum involved in decoding the behaviorally contingent population responses of cholinergic interneurons.

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VISITOR INFORMATION

EMERGENCY	CSHL	BANBURY
Fire	**(9) 742-3300**	**(9) 692-4747**
Ambulance	**(9) 742-3300**	**(9) 692-4747**
Poison	**(9) 542-2323**	**(9) 542-2323**
Police	**(9) 911**	**(9) 549-8800**
Safety-Security	**Extension 8870**	

Emergency Room **Huntington Hospital** 270 Park Avenue, Huntington	**631-351-2300** **(1037)**
Dentists Dr. William Berg Dr. Robert Zeman	 **631-271-2310** **631-271-8090**
Doctor MediCenter 234 W. Jericho Tpke., Huntington Station	**631-423-5400** (**1034**)
Drugs - 24 hours, 7 days Rite-Aid 391 W. Main Street, Huntington	**631-549-9400** (**1039**)

Free Speed Dial
Dial the four numbers (********) from any **tan house phone** to place a free call.

GENERAL INFORMATION

Books, Gifts, Snacks, Clothing, Newspapers
BOOKSTORE 367-8837 (hours posted on door)
Located in Grace Auditorium, lower level.

Photocopiers, Journals, Periodicals, Books, Newspapers
Photocopying – Main Library
Hours: 8:00 a.m. – 9:00 p.m. Mon-Fri
10:00 a.m. – 6:00 p.m. Saturday
Helpful tips - Obtain PIN from Meetings & Courses Office to enter Library after hours. See Library staff for photocopier code.

Computers, E-mail, Internet access
Grace Auditorium
Upper level: E-mail only
Lower level: Word processing and printing.
STMP server address: mail.optonline.net
To access your E-mail, you must know the name of your home server.

Dining, Bar
Blackford Hall
Breakfast 7:30–9:00, Lunch 11:30–1:30, Dinner 5:30–7:00
Bar 5:00 p.m. until late
Helpful tip - If there is a line at the upper dining area, try the lower dining room

Messages, Mail, Faxes

Message Board, Grace, lower level

Swimming, Tennis, Jogging, Hiking

June–Sept. Lifeguard on duty at the beach. 12:00 noon–6:00 p.m. Two tennis courts open daily.

Russell Fitness Center

Dolan Hall, west wing, lower level
PIN#: **Press 64300 (then enter #)**

Concierge

On duty daily at Meetings & Courses Office.
After hours – From tan house phones, dial x8870 for assistance

Pay Phones, House Phones

Grace, lower level; Cabin Complex; Blackford Hall; Dolan Hall, foyer

CSHL's Green Campus

Cold Spring Harbor Laboratory is pledged to operate in an environmentally responsible fashion wherever possible. In the past, we have removed underground oil tanks, remediated asbestos in historic buildings, and taken substantial measures to ensure the pristine quality of the waters of the harbor. Water used for irrigation comes from natural springs and wells on the property itself. Lawns, trees, and planting beds are managed organically whenever possible. And trees are planted to replace those felled for construction projects.

Two areas in which the Laboratory has focused recent efforts have been those of waste management and energy conservation. The Laboratory currently recycles most waste. Scrap metal, electronics, construction debris, batteries, fluorescent light bulbs, toner cartridges, and waste oil are all recycled. For general waste, the Laboratory uses a "single stream waste management" system, removing recyclable materials and sending the remaining combustible trash to a cogeneration plant where it is burned to provide electricity, an approach considered among the most energy efficient, while providing a high yield of recyclable materials.

Equal attention has been paid to energy conservation. Most lighting fixtures have been replaced with high efficiency fluorescent fixtures, and thousands of incandescent bulbs throughout campus have been replaced with compact fluorescents. The Laboratory has also embarked on a project that will replace all building management systems on campus, reducing heating and cooling costs by as much as twenty-five per cent.

Cold Spring Harbor Laboratory continues to explore new ways in which we can reduce our environmental footprint, including encouraging our visitors and employees to use reusable containers, conserve energy, and suggest areas in which the Laboratory's efforts can be improved. This book, for example, is printed on recycled paper.

1-800 Access Numbers

AT&T	**9-1-800-321-0288**
MCI	**9-1-800-674-7000**

Local Interest

Fish Hatchery	631-692-6768
Sagamore Hill	516-922-4447
Whaling Museum	631-367-3418
Heckscher Museum	631-351-3250
CSHL DNA Learning Center	x 5170

New York City

Helpful tip -

Take Syosset Taxi to Syosset Train Station ($8.00 per person, 15 minute ride), then catch Long Island Railroad to Penn Station (33rd Street & 7th Avenue). Train ride about one hour.

TRANSPORTATION

Limo, Taxi

Syosset Limousine	516-364-9681 (**1031**)
Super Shuttle	800-957-4533 (**1033**)
To head west of CSHL - Syosset train station	
Syosset Taxi	516-921-2141 (**1030**)
To head east of CSHL - Huntington Village	
Orange & White Taxi	631-271-3600 (**1032**)
Executive Limo	631-696-8000 (**1047**)

Trains

Long Island Rail Road	822-LIRR
Schedules available from the Meetings & Courses Office.	
Amtrak	800-872-7245
MetroNorth	800-638-7646
New Jersey Transit	201-762-5100

Ferries

Bridgeport / Port Jefferson	631-473-0286 **(1036)**
Orient Point/ New London	631-323-2525 **(1038)**

Car Rentals

Avis	631-271-9300
Enterprise	631-424-8300
Hertz	631-427-6106

Airlines

American	800-433-7300
America West	800-237-9292
British Airways	800-247-9297
Continental	800-525-0280
Delta	800-221-1212
Japan Airlines	800-525-3663
Jet Blue	800-538-2583
KLM	800-374-7747
Lufthansa	800-645-3880
Northwest	800-225-2525
United	800-241-6522
US Airways	800-428-4322