

Harvard University FAS Molecular and Cellular Biology

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Genetic programs downstream of Ctip2 regulating development of corticospinal motor neurons and medium spiny neurons

Abstract

The complex neural networks responsible for processing of sensory stimuli, and controlling cognition and motor responses in the mammalian neocortex are composed of diverse and functionally specialized neuronal subtypes generated during development under the precise control of molecular and genetic programs. Corticospinal motor neurons (CSMN) are an important neocortical projection neuron subtype that controls voluntary motor behavior. CSMN degenerate in motor neuron diseases such as amyotrophic lateral sclerosis (ALS), hereditary spastic paraplegia (HSP), and primary lateral sclerosis (PLS), and damage to their axons is central to spinal cord injury.

In recent years, as genetic and molecular controls over the generation and maturation of CSMN have begun to be elucidated, one transcription factor, Ctip2 (COUP-TF interacting protein 2), has emerged as a central control over CSMN differentiation. Ctip2 function in CSMN is critical for their axon outgrowth, path finding, and fasciculation. In addition, Ctip2 controls the differentiation of medium spiny neurons (MSN) in the striatum, a basal forebrain structure through which CSMN axons travel on their trajectory to the spinal cord. In Ctip2 null mice, CSMN axon projections are disorganized throughout the forebrain, fasciculate poorly as they course through the striatum, and fail to reach the spinal cord. While cell-autonomous Ctip2 function has been demonstrated to be responsible for some aspects of CSMN axon extension to the spinal cord, it is not known whether loss of Ctip2 function in MSN also contributes to abnormalities in CSMN axon development.

In this thesis, I investigate non-cell-autonomous mechanisms by which Ctip2 functions in MSN to facilitate fasciculation and outgrowth of CSMN axons. Molecular and anatomical analysis of a novel *Gsx2-Cre;Ctip2^{fl/fl}* conditional null mutant mouse line, in which Ctip2 expression is absent specifically in MSN, reveals that without Ctip2 function in MSN, CSMN axons are defasciculated in the internal capsule, and few reach the spinal cord. Furthermore, I find that targeted deletion of Ctip2 in MSN disrupts development of the striatum, suggesting that CSMN-extrinsic factors in the striatal environment are critical for CSMN axon connectivity. Using RT-PCR, in situ hybridization, and immunocytochemistry to compare gene expression in wild-type, Ctip2⁻ null, and *Gsx2-Cre;Ctip2^{fl/fl}* conditional null striatum, I have identified novel gene candidates downstream of Ctip2 in the striatum (including *Adora2*, *Drd1a*, and *Efnb3*), whose developmental expression is dramatically disrupted in the absence of Ctip2. These candidate genes are potentially instructive for establishment of proper striatal cellular architecture and for CSMN axon fasciculation. Through ongoing in vivo overexpression and/or knockdown experiments, I will more deeply investigate functions of individual gene candidates in CSMN axon outgrowth and fasciculation. Together, these results indicate that Ctip2 regulates a program of molecular signals in MSN that controls not only development of MSN, but also controls non CSMN-autonomous fasciculation of CSMN axons in the internal capsule and extension of these axons toward the spinal cord.

A Psychophysical Analysis of contrast sensitivity through visual change detection behavior in the mouse

Abstract

Due to the rise of genetic approaches to systems neuroscience, the mouse has gained tremendous popularity as a model organism. With appropriate behavioral assays, many questions about the relationship between electrical activity and behavior as well as its underlying circuitry could be addressed in the mouse with powerful genetic and optical approaches. In particular, the mouse visual system is well suited for these studies because of the rigorous stimulus control available. Neurons in mouse visual cortex show orientation and contrast tuning similar to primates. However, the lack of precise behavioral assays to measure these properties is a limiting factor in pertinent studies of cortical coding and behavioral readout. To address this problem, we have developed a head-fixed behavioral task involving the manipulation of a lever to report the detection of visual stimuli and shown that psychophysical measurements can be robustly obtained. We have demonstrated that we can get mice to work consistently at such a task for hundreds of trials daily and that we can collect repeatable psychophysical thresholds of contrast sensitivity from multiple animals that remain stable over time. Our detection thresholds are more sensitive than those obtained using optokinetic responses, demonstrating the great potential of this method for use in both psychophysical data collection as well as the study of cortical coding in the mouse.

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Spatial Organization of Neocortical Myelinated Axons at the Ultrastructural Level

Abstract

Myelinated axons are long neuronal processes extending up to meters in length and are involved in the long-distance communication in the brain circuitry. While the gross architecture has been well studied, the organization of myelinated axons at a microscopic (ultrastructural) level remains unclear. In a volume of mouse neocortex imaged using electron microscopy, trajectories of nonfasciculated myelinated axons are observed to intersect and form a crisscross pattern. At the points of intersection, myelinated axons appear tightly apposed to one another. The close apposition of myelinated axons might be a consequence of a developmental mechanism. If so, these appositions might provide insight into the way in which tissue expansion modifies the pattern of neural wiring. Indeed, the appositions might be anchoring junctions maintained by the cell adhesion molecules and form the basis of a scaffolding framework that supports the structural integrity of the entire neuropil. A scaffold of myelinated axons could contribute to stability in the arrangement of neural elements and potentially reveal a novel organizational principle for the structure of brain circuits.

If there is a spatial organization among myelinated axons, then the number of touches observed in the volume should be significantly higher than the number expected from a random arrangement of myelinated axons. To test if there is nonrandom organization, myelinated axons were modeled geometrically in several different ways and Monte Carlo simulations were designed to compute the probability that randomly positioned myelinated axons form at least the observed number of appositions. Some of the results show that the observed number of appositions is expected of random arrays of myelinated axons. These results suggest that trajectories of myelinated axons are uncorrelated with respect to each other and that a potential scaffold of myelinated axons would be based on axons that are randomly organized during development.

Neuropeptide function in larval zebrafish: The Role of Cholecystokinin in anxiety-related behavior

Abstract

Neuropeptides have been shown to be important modulators in a wide variety of behaviors. One behavior in which neuropeptides are poised to play a role is anxiety. An understanding of the molecular mechanisms underlying anxiety is essential for designing effective and targeted treatments for anxiety disorders. Zebrafish have emerged as a powerful system in which to study behavior, with the ability to investigate function at the level of genes, neuroanatomy, and neural circuits. My primary objective in this project was to determine whether larval zebrafish could be used to study anxiety-related behavior, thereby leveraging the strengths of larval zebrafish to overcome limitations in existing rodent and adult zebrafish systems. Specifically, I sought to elucidate the basal anxiety-related phenotypes in a vertebrate with a relatively simple nervous system. To do so, I used genetic and pharmacological tools that were previously known to have either anxiogenic or anxiolytic effects. As a test case for more detailed analysis, I focused on cholecystokinin (Cck), a neuropeptide previously shown to have anxiogenic activity. Through expression analysis, I showed that the neuroanatomy of the Cck system in larval zebrafish, found in areas such as the amygdala homolog and the habenula, poises it to influence anxiety. Furthermore, I found that in larval zebrafish, Cck increased both general locomotor activity and response to stressor stimuli, including light and heat stimuli. This work shows that larval zebrafish do indeed display behavioral phenotypes in response to anxiogenic or anxiolytic molecules and therefore have the potential to be used as a high-throughput model to investigate modulators of anxiety-like behavior.

Visualizing TRN synchrony: Developing a protocol for studying gap junction coupled cells and their plasticity

Abstract

Historically, the role of electrically coupled neurons in the mammalian CNS has been largely overlooked. In the thalamic reticular nucleus (TRN), however, gap junction coupled pairs are quite prevalent. Because the TRN is responsible for modulating thalamocortical signals and has been implicated in inducing irresponsiveness to sensory input during deep sleep and spike-wave seizures, it is important to characterize the role played by gap junction-coupled cells within the TRN. In order to study the activity of multiple cells within a gap junction-coupled network, I developed a protocol that goes beyond traditional patch-clamping techniques. First, I explored ways to correlate the electrophysiological data acquired during whole-cell recordings with the activity in the highly noisy fluorescence data acquired by bathing the tissue in the calcium sensitive dye Oregon Green Bapta (OGB). These experiments, as well as later experiments involving the PKA inhibitor KT5720, revealed that calcium-sensitive imaging is a dynamic way of studying gap-junction coupling amongst a population of neurons and, more importantly, studying plasticity of coupling. Also, by experimenting with calcium concentrations, I showed that TRN cells begin firing more bursts in conditions of low calcium and that these bursts induce firing in as many as 8 other TRN cells via gap junctions. These results indicate that calcium signaling may be involved in producing the changes to the TRN that lead to synchronized oscillations. More importantly, our experiments show that gap junctions play an important role in coordinating TRN synchrony during the high burst activity periods associated with deep sleep and spike wave-seizures.

Characterization of Dopaminergic Neurons in the Rodent Olfactory Bulb in vivo

Abstract

The vertebrate olfactory bulb (OB) contains dopaminergic (DA) neurons that mediate diverse functions. These functions include discrimination of various odors and dynamic sensory processing of odorant information. OB DA cells, which are primarily periglomerular, receive innervations from the olfactory receptor neurons and modulate sensory information output. In the present study, we measured odor-evoked calcium responses by bulk-loading the OB with acetoxymethyl ester of the synthetic calcium dye Oregon Green Bapta. The DA cells were identified by using a transgenic mouse expressing a red fluorescent protein driven by the promoter of the dopamine active transporter (DAT:dTomato). Using two-photon microscopy, we successfully imaged odor-evoked activity of these DA cells in vivo while the mouse is freely breathing under anesthesia. The DA phenotype of genetically labeled DAT:dTomato cells were confirmed by immunostaining fixed brain slices with tyrosine hydroxylase (TH), the first enzyme in the DA biosynthetic pathway. We found some discrepancies in cells identified genetically versus labeled by TH antibody. We believe the discrepancies can be attributed to neurogenesis and/or the "fate-switching" nature of DA cells in the rodent OB. Further study is needed to determine how these DA PG neurons differ from other PG cells, such as the PG cells expressing only GABA, and how DA plays a unique role in the modulation of sensory information output.

**Relations Between Associative Processing and Mood: Behavioral and Neuroimaging
Evidence in Patients with Major Depressive Disorder and Healthy Controls**

Abstract

Bar (2009) proposed that there exists a direct and reciprocal relationship between associative processing and mood, whereby positive mood improves associative thinking ability and broadly associative thinking elevates mood. Bar hypothesizes that patients with major depressive disorder (MDD) have impaired associative processing ability since they are prone to increased rumination, a persistently self-focused and narrow thought process. We investigate the proposed framework using two different approaches. Experiment 1 examined whether the scope of an individual's unrestrained associative processing could induce a change in mood. We tested this by asking subjects to orally produce a chain of associated words for duration of ten minutes. As hypothesized, the results showed that subjects who demonstrated broader associative thinking ability, as evidenced by more rapid and fluid transitions between contextual frames, experienced improvements in positive mood. Experiment 2 investigated morphological abnormalities in MDD patients by analyzing six brain regions central to the associative processing network: the hippocampus, medial orbitofrontal cortex, entorhinal cortex (ERC), amygdala, parahippocampal cortex, and the nucleus accumbens (NAcc). Our results revealed significantly enlarged ERC and NAcc in MDD patients compared to healthy controls. Further analysis showed that ERC volume differences were significantly larger in chronic MDD patients than in recurrent MDD patients. The results also indicated that the duration since the first onset of illness was a statistically significant predictor of ERC volume differences. Although not directly evidenced by our results, we speculate that hyperactivity of the ERC could augment the release of glutamate, thereby inducing the hippocampal atrophy typically found in MDD.

Spatial Discounting in the Domestic Dog: Examining a novel behavioral model system for economic decision making

Abstract

The human tendency to devalue rewards over time, known as temporal discounting, has been studied in a variety of animals ranging from pigeons to marmosets. But while these model systems help elucidate certain mechanisms of temporal discounting, the applicability of intertemporal choices for animal systems remains problematic in terms of design complexity and evolutionary saliency. Instead, I propose to explore the efficacy of a possible spatial discounting paradigm that requires no learning and is more ecologically natural for animals. Specifically, I tested to see if domestic dogs are influenced by distance cues in choosing between reward distributions. By conducting a series of 2-choice paradigms across various distances, I found that: dog choice of food rewards (FR) varied significantly with relative proximity of food rewards ($p < .001$ in all cases); the relative distance between FR's (both as a ratio to the subject and absolute difference in distance) significantly influenced subjects correctly choosing some FR over no FR (Exp 1. $P = .039$); and this effect increased with introduction of various numbers (more FR over less FR, Exp 2, $p = .018$). Furthermore, subject preference reversal due to ratio effects of distance are similar to ratio effects seen in hyperbolic temporal discounting of pigeons and follow Weber's law of magnitude ratio. Finally, success in Exp. 1 correlated with success in Exp 2 ($p = .298$, $p = .027$); number discrimination ability also correlated with Exp 2 ($p = .281$, $P = .037$). While this is only a preliminary study of dogs' capacity to use distance in making food choices, the results tentatively validate the methodology and suggest that a putative canine spatial discounting process might stem from a combination of separate distance and number discriminating abilities.

Connectivity between motor Learning-Associated Brain areas in the rat: an interactive map

Abstract

Previous research in human subjects and other primates has implicated the primary motor cortex, several premotor areas, striatum, and prefrontal cortex as areas involved in the acquisition of motor sequences. However, to better formulate hypotheses about the structure of the circuits involved in motor learning, a good understanding of the underlying connectivity between these areas is needed. The work for this thesis consists of a literature review of the known connections between regions putatively involved in motor learning and the construction of an interactive visual map of these areas as a future reference for the lab. It also comprises anterograde and retrograde tracing studies demonstrating connectivity between the rostral and caudal forelimb areas in the codex, and from the caudal forelimb area in the primary motor cortex to the striatum. For making the interactive map, I tried several approaches. First, I tried making a diagram in Adobe Illustrator, but this turned out to be unable to give a clear picture of connections as the diagram became too complex to be read easily. Second, a database written in Matlab script could save relevant connectivity information about areas, but it lacked a visual component that would make the map intuitive and easy to browse. Finally, the map was implemented as a Python script that could be visualized in the Neuroptikon software developed at Janelia Farm. For the tracing studies, areas of the motor cortex controlling movement of the contralateral forelimb were located functionally using intracortical microstimulation of about 200 pA in the output layer V under isoflurane or ketamine anesthesia. The sensorimotor striatum was approximated using its coordinates in an anatomical rat atlas and confirmed later with histology. An anterograde tracer was injected at the source and a retrograde tracer was injected at the target of a putative connection. Following a period of 7-10 days for neuronal uptake and transport, rats were perfused with paraformaldehyde, and brain tissue was sectioned after at least one day of post-fixings. The fixed tissue was photographed on a confocal microscope. Tracing experiments confirmed some connections that have been demonstrated in previous papers between the caudal motor area (which is thought to correspond to the primary motor cortex) and the rostral forelimb area (which is thought to correspond to the supplementary motor area and/or the premotor area of other mammals), but failed to replicate others.

Using the RGC-5 cell model for the study of RGC development and regeneration

Abstract

Retinal ganglion cells (RGCs) are the neurons whose axons comprise the optic nerve, and are destroyed in diseases such as glaucoma, leading to blindness. Although RGCs are not able to regenerate in the adult mammalian environment, RGCs regenerate successfully in frogs and goldfish, restoring functional vision. Furthermore, RGCs can regenerate after injury in the embryonic mouse, but this ability is lost approximately two days before birth. Use of primary cultured RGCs for study presents several problems, such as difficulty in purifying homogenous cell populations and difficulty in gene transfection and manipulation. The RGC-5 cell line, a transformed mouse RGC line, addresses these problems and provides a potential model to study the genetic programs involved in axon regeneration. However, RGC-5 is a recently developed cell line which has not yet been extensively studied. My project aims to characterize the RGC-5 cell line to determine the optimal conditions for which it can serve as a sufficient model of RGC development. RGC-5 can be differentiated with staurosporine to resemble RGC morphology, developing a rounded soma and extending neurites. The optimal differentiation condition was found to be 316 nM staurosporine at a cell density of 45,000 cells/mL. Much of differentiation was discovered to occur in the hour immediately following exposure to staurosporine, with upregulation of neuronal markers such as Map2ab, Thy 1.2, Tau, Brn3b, and beta-3 tubulin, confirming RGC-5's viability as an RGC model. Furthermore, IGF-1 was found to be dramatically upregulated immediately after differentiation, concurrent with neurite growth, providing further evidence for its importance in axonal growth.

Effect of APOE Allelic Variation on Default Network in Young Adults

Abstract

Apolipoprotein E e4 allele (APOE e4) is a major genetic risk factor for Alzheimer's disease. Current research has focused much on e4's effects on the elderly. However, recent studies suggest that e4 may produce individual differences early in life that lead to predisposition for AD. This study sought to test the hypothesis that cognitively normal young adult e4 carriers (ages 18- 35) show significant functional and structural differences than their respective non-carriers. Data for the experiment was obtained from a large-scale repository of human genetic and neuroimaging data. 357 Caucasians and 70 Asians aged 18-35 were chosen for testing. After first partialing out variance associated with gender, age, and handedness, e4 carriers and non-carrier controls in each ethnicity were compared on amygdala and hippocampal volume and default network strength using t-tests. Caucasians were also compared on cortical thickness. We found no statistical difference in the default network correlations, amygdala volumes, and hippocampal volumes between e4 carriers and non-carriers in either of the ethnic groups. No significant differences were seen in cortical thickness between the e4 carriers and non-carriers in Caucasian subjects. The results suggest that if e4 does exert effects early in life, they are too subtle to be detected using current measures. The e4 allele's contribution to AD risk via neurodevelopmental pathway is likely small. We believe this study is an important first step in understanding functional properties related to AD risk very early in life, possibly, aiding in development of better therapeutics.

Characterizing the Ethological Function of Individual Vomeronasal Receptors

Abstract

Sensory stimuli serve as principal determinants of social behavior in many organisms. In mice, pheromone signaling conveys such information as gender, social status, and organism identity, yet the roles and information coding by neurons of one pheromone-sensing system, the vomeronasal organ (VNO), remain largely unknown. Here, we report development of a large set of vomeronasal receptor probes that can distinguish between individual vomeronasal sensory neuron types and their use in analyzing the response properties of VSNs to social cues from other organisms. We describe the characterization and implementation of a system to exogenously activate VSNs in transgenic subjects expressing artificial cation channels. RNA in situ hybridization techniques detecting neuronal activity through the expression of immediate early genes detected a wide class of heterospecific chemosignals by the VNO. We also uncovered the molecular logic of chemosignal encoding in the VNO for V1Rc and V1Rh receptors, both at the clade level and at the level of the individual receptor. The artificial activation of VSNs by chimeric receptors in our transgenic system was reported, yet the neuronal responses generated by artificial receptors were found to be weaker in comparison to the responses reported for natural VSN ligands. The association of single vomeronasal receptors with ethologically relevant cues, in concert with the characterization of a system for artificially activating VSNs in vivo, provides a potential mechanism for investigating the contribution of individual vomeronasal receptors to behavior.

Comprehensive Identification of direct inputs into midbrain Dopamine Neurons using modified rabies virus.

Abstract

The midbrain dopamine system plays a pivotal role in regulating motivation, learning, and movement. Malfunction of this system can result in various devastating disorders such as Parkinson's disease and schizophrenia. Developing a better understanding of the neural circuits that regulate dopamine neuron activity will thus provide critical insight into how circuits underlie behavior, various disorders, and therapeutic approaches. Such efforts have been hindered by our limited knowledge regarding the connectivity of specific types of neurons within a circuit. In order to resolve this problem, we modified the rabies virus-mediated retrograde transsynaptic tracing technology so that starter cells can be restricted to a specific neuron type. Using this technique, we identified monosynaptic inputs into dopamine neurons in the ventral tegmental area (VTA), substantia nigra pars compacta (SNc), and striatum.

Comprehensive input mapping revealed several novel connections in addition to previously reported connectivity. Notably, several areas exhibited preferential connections to either the VTA or SNc. Among novel connections, we show that the subthalamic nucleus (STN) a major target for deep brain stimulation for Parkinson's disease, directly to dopamine neurons in the SNc preferentially. Based on this finding, we propose that the effects of deep brain stimulation may be due in part to increased activity of SNc dopamine neurons, which leads to restoration of dopamine levels in the target areas. The connectivity diagram obtained in this study will provide important insights into the functions of the VTA and SNc dopamine systems as well as the mechanisms of deep brain stimulation.

The optokinetic response and horizontal cell connectivity of the nof mutant zebrafish

Abstract

Zebrafish (*Danio rerio*) are a useful model organism for investigating the vertebrate retina. A mutant strain known as no optokinetic response f (nof) has nonfunctional cones due to a mutation in the coding of cone transducin α , but research shows that there is a transducing independent Ca^{2+} -mediated response to bright light conditions. My research investigates whether or not this transducin-independent response can drive visual behavior. Furthermore, I examine the question of whether functional cones are necessary for either the correct synaptic wiring of cone photoreceptors to horizontal cells or for the maintenance of these synapses over time. Zebrafish offspring were screened at 5 days post-fertilization (dpf) to separate the nof mutants from sighted age-matched controls. Both groups were tested for OKR under a variety of light conditions and motion speeds. Once in adulthood, retinas were whole-mounted and stained with DiI inserted as a powder on the tip of a glass micropipette. These retinas were imaged with a confocal microscope to compare their horizontal cells with those of age-matched sighted controls. The nof mutant fish continued to exhibit a negative OKR under all test conditions, while sighted control fish retained a positive OKR. The horizontal cells in the retinas of adult nof fish retained the same connectivity patterns as horizontal cells in wild-type fish. Nof mutant fish H1 cells made fewer connections and had smaller synapses than control siblings. nof mutant H3 cells had slightly larger dendritic fields and made more connections than control fish H3 cells. Additionally, nof mutant H3 cells had soma that were less compact and rounded as compared to control H3 cells. These findings support the conclusion that the transducin-independent bright light response is inadequate to drive visual behavior. Additionally, functional cones are not necessary for specific synaptic wiring of cones to horizontal cells, but the loss of function in cone transducin does have specific effects on different horizontal cell populations in different ways.

A role for the Ubiquitin Proteasome System in Modulating the Strength of Electrotonic Synapses in the Mammalian Thalamus

Abstract

Plasticity at neural synapses in the central nervous system is essential for proper cognitive function in mammals. The most widely studied forms of plasticity occur at chemical synapses in the brain; however, recent studies have shown that plasticity can also occur at electrotonic synapses formed by gap junction channels linking the cytosols of two neighboring neurons. However, the molecular details behind these changes in signaling strength remain unknown. This thesis addresses the role of the ubiquitin proteasome system as a cellular process underlying plasticity at gap junctions in mammalian thalamic neurons. Dual, whole-cell recordings from slice preparations of neurons in the thalamic reticular nucleus were made to assess the coupling strength of two neurons coupled by gap junctions. Pharmacological blockers of proteasome activity were then used to assess the role of the proteasome in modulating the coupling strength at electrotonic synapses. Similar to gap junctions in other tissue types, proteasome inhibition increased the coupling strength of neurons coupled by electrotonic synapses. In addition, possible asymmetries in directional coupling between paired neurons were identified. These results suggest a potential role for the proteasome in mediating plasticity at electrotonic synapses in the brain, similar to what has previously been shown for plasticity at gap junctions in other cell types as well as some forms of plasticity at chemical synapses.

Investigating the role of 5-HT1B serotonin receptors in aggression in *Drosophila melanogaster*

Abstract

Aggression is a complex, innate social behavior that is intricately tied to the nervous system. While both invertebrate and vertebrate animal models of aggression have consistently highlighted the importance of brain serotonin (5-HT) in this behavior, the mechanisms by which serotonin exerts its influence are not well understood. Pharmacological manipulation of the 5-HT_{1A} serotonin receptor subtype in mice and rats has yielded a behavioral phenotype of decreased aggression. Given the complexities of the mammalian nervous system and the indirect pharmacological approach previously employed, I decided to use the *Drosophila melanogaster* model of aggression to further investigate the targets of serotonergic action in the nervous system. The closest ortholog of the mammalian 5-HT_{1A} receptor in *Drosophila melanogaster* is the d5-HT_{1B} serotonin receptor subtype. In order to clarify the role of this receptor in aggressive behavior, I have used the Gal4/UAS system to generate fruit flies with altered expression of the 5-HT_{1B} gene and have tested these flies for changes in aggression. Flies that were manipulated to have chronic overexpression of 5-HT_{1B} receptors display a slight hyper aggressive tendency, exhibit abnormal male-male courtship behavior during aggression assays, and have significantly longer encounters in fights. Short-term activation of the 5-HT_{1B} circuitry does not produce any abnormal aggression or courtship behavior, suggesting that the male-male courtship phenotype seen with chronic overexpression is due to a developmental defect. Finally, an RNAi silencing approach to shut down the expression of this receptor type does not yield any effect on aggression, though the efficiency of the genetic tool remains to be investigated. These data suggest that chronic enhancement of the response properties of certain 5-HT sub-circuits in *Drosophila melanogaster* may lead to a mixing of courtship and aggression during male-male behavioral interactions.

Assessing the therapeutic and side-effect profiles of novel antipsychotic drugs through pre-pulse inhibition of startle and operant conditioning rate assay experiments

Abstract

Pre-pulse inhibition (PPI) of the startle response is an unconditioned neurological reaction that reflects the adaptability of the nervous system to external cues and warnings. When an organism is exposed to a low-intensity pre-stimulus 30-500 milliseconds prior to a high-intensity startling stimulus, the startle response to the strong stimulus is reduced, reflecting normal sensorimotor gating. PPI is an important phenomenon and research tool for the following reasons: First, patient populations with certain psychiatric disorders, including schizophrenia, exhibit deficits in PPI. Second, although experimentally induced PPI deficient rodents are not animal models of diseases such as schizophrenia per se, all widely prescribed antipsychotic medications improve PPI, and the paradigm is widely used to assess the efficacy of potential drug treatments for schizophrenia. This thesis aimed to assess the therapeutic and side-effect profiles of antipsychotic drugs. First, the ability of antipsychotic drugs to recover PPI in experimentally induced PPI deficient rodents was evaluated to determine the therapeutic effects. Next, the side-effects profile of these drugs was determined by recording the effects of the same drugs on the motor response rate of normal rats trained in a food maintained operant behavior task.

To create a PPI-deficient rodent, apomorphine, a dopamine receptor agonist, was used. Next, we assessed the ability of a novel dopamine D2 receptor antagonist, CTDP 30,852, to recover PPI. We found that this compound did not recover the apomorphine-induced deficit of PPI. On the other hand, the dopamine D2 antagonist haloperidol did appear to dose-dependently recover the PPI deficit, though the results did not reach statistical significance. The test for motor side-effects revealed that CTDP 30,852 had no effect on the rate of response of rats 5 trained on a food maintained operant behavior task, even at the highest dose, while haloperidol dose-dependently decreased the rate of response.

Interestingly, because CTDP 30,852 does not bind to sigma receptors whereas haloperidol does, an additional drug was evaluated to test the hypothesis that sigma receptor binding may be partly responsible for motor side effects. When administered alone, the sigma receptor agonist, DTG, resulted in a modest dose-dependent recovery of PPI. This novel finding warrants further investigation in order to evaluate sigma receptors as a potential target for novel antipsychotic drugs with fewer side effects.

Hey, that's not my bed!: The effects of sleep environment on sleep-dependent memory consolidation

Abstract

Objective: Determine the effect of sleep environment on (i) overnight improvement on a visual texture discrimination task, and on sleep, measured (ii) objectively and (iii) subjectively. **Methods:** 24 healthy, college-aged subjects (n=16 male) participated in a three-part, repeated measures sleep study. Subjects arrived in the evening at Beth Israel Hospital and were trained on the visual discrimination task (VDT); they then slept in one of three locations in randomized order: a) at Home b) in a spacious Lab-furnished room or c) on a Hospital ward; after 8 hours of sleep opportunity, subjects were tested on the VDT. Polysomnographic (PSG) recordings were taken only in the Lab and Hospital. **Results:** VDT: Performance showed significant (8.0%) overnight improvement when subjects slept at Home, differing significantly from the Hospital night (-0.2% ; Home vs. Hospital: $p < .02$); VDT performance showed improvement in the Lab (2.8%), but it was not significantly different from the Home or Hospital. PSG: Sleep efficiency was significantly higher ($p = .025$) for subjects sleeping in the Lab (94.6%) than in the Hospital (89.5%), with a loss of REM in the Hospital. Questionnaires: In contrast to PSG data, subjects largely reported better sleep in the Hospital than in the Lab. **Conclusions:** Analyses of these data suggest (i) overnight sleep-dependent improvement is sensitive to sleep environment, (ii) poorer objective sleep quality correlates with less overnight improvement, both between subjects in a given environment, and between environments for individual subjects, and (iii) subjective sleep quality predicts objective sleep in the Lab, but not the Hospital.

Impact of chronic sleep deprivation on pain thresholds in the mouse

Abstract

Complaints about pain and non-restorative sleep (increased arousal, sleep fragmentation) are very often expressed by the same patients, indicating a possible link between sleep and pain. However, little is known about the possible mechanisms underlying the reciprocal interaction between pain and sleep, and animal data are scarce. The main goal of this project was to investigate in mice the effects of sleep restriction on pain sensitivity. As chronic sleep loss is more likely to alter pain perception in humans, we developed a novel protocol of chronic total sleep restriction in mice. Mice were sleep deprived 6 hours per day for 5 consecutive days from the onset of the light period using gentle, minimally-stressful procedures. The animals were instrumented for sleep (EEG/EMG electrodes) to confirm the full extent of the sleep deprivation and to analyze its consequences on sleep homeostasis. Pain thresholds for thermal (hotplate at 52C) and mechanical (von Frey filaments) modalities were assessed after the first, third and fifth sessions of sleep deprivation. We demonstrated that chronic sleep deprivation led to decreased pain thresholds, with a maximum effect at day 5. These data strengthen the hypothesis of a direct relationship between sleep homeostasis and the processing of painful stimuli and warrant future studies to tease out its exact nature.

Inosine enhances anatomical reorganization and functional outcome following spinal cord injury

Abstract

The principal objective of this thesis was to test the hypothesis that the nucleoside inosine enhances anatomical reorganization of the rat spinal cord following a transection of the corticospinal tract (CST) and other pathways. We proposed the mechanism of this reorganization was that inosine promotes sprouting in the CST, resulting in the formation of functional synapses with the propriospinal tract (PST), thus creating a new circuit between the brain and hindlimbs. Testing this hypothesis included creating four groups of animals with different combinations of either a dorsal hemisection surgery or not, and osmotic minipumps delivering either inosine or saline. Behavioral testing included weekly ladder walk analysis and scoring based on a standardized locomotor testing scale. Tissue analysis included: bouton quantification using biotinylated dextran amines (BDA)/cholera toxin B (CTb) tracers and BDA/choline acetyltransferase (ChAT) tracers, and serotonin (5-HT) nerve terminal sprouting analysis. We found that behaviorally, rats that underwent the surgery and that were treated with inosine regained coordination and correct limb function more quickly and to a fuller extent than rats having undergone the same surgery but treated with saline. Inosine-treated animals that underwent surgery also showed significant serotonergic nerve terminal increase over their saline counterparts. When boutons were counted, the experimental group showed evidence for increased sprouting and synapses over the control groups. These results suggest that inosine shortens the required time for behavioral recovery from spinal cord injury. Furthermore, tissue analysis suggests that improved sprouting is the anatomical mechanism underlying improved recovery.

Neural correlates of paired associate learning in development

Abstract

The purpose of this study was to clarify the role of the hippocampus in learning during development, and to determine the extent to which other neural regions drive observed developmental differences in hippocampal function. Our study targeted the hippocampus by using a paired associate learning paradigm during fMRI imaging in children ages 8-11 and adults. A region of interest analysis was conducted to examine differing patterns of activation among 8-9 year olds, 10-11 year olds, and adults. While 10-11 year olds and adults showed greater activation to binding pairs rather than individual items, 8-9 year olds showed less differential activation, suggesting a developmental shift in hippocampal function. We then asked whether this progression in hippocampal function was due to changes in connectivity with the prefrontal cortex. A connectivity analysis was conducted to see if 10-11 year olds and adults still shared similar emerging networks. While adults had significant connectivity between dorsolateral prefrontal cortical and medial temporal lobe areas, 8-9 and 10-11 year olds did not. When connectivity from the medial temporal lobe to all regions of the brain was examined, adults showed significant connectivity between the hippocampus and a temporopolar area, while children showed only connectivity within medial temporal lobe regions. This suggests that the developmental shift in hippocampal function between 8-9 and 10-11 years is not due to changes in connectivity with the prefrontal cortex, and may be a result of maturation of the hippocampus alone.

The iPS Connection: Bridging the Gap Between Alzheimer's Disease Research and Clinical Disease Pathology with a Novel, Induced Pluripotent Stem Cell-based Assay of A secretion

Abstract

Alzheimer's disease (AD) is a debilitating neurodegenerative disease that carries substantial morbidity and economic burden for millions worldwide. The pathogenesis of AD has been linked to beta-amyloid (A β), a protein secreted from neurons that has been shown to be the primary component of the hallmark plaques that develop in the brains of AD patients. Cleavage of amyloid precursor protein (APP) gives rise to A β , and rare mutations in the gene encoding APP have been linked to early-onset Alzheimer's disease. A secretion has been studied using a number of model systems, post-mortem biopsies of AD patients, and indirect measurements of A secretion (e.g. in blood plasma); however, there has been little opportunity to fully characterize A production directly from living human neurons. Thanks to recently developed techniques, it is now possible to derive pluripotent stem cells that share the genetic makeup of the fully differentiated cells they are derived from. Reprogramming these induced pluripotent stem cells (iPSCs) to a neuronal fate opens new avenues into understanding the mechanisms of neurodegenerative diseases like AD. We developed an *in vitro* assay for A secretion from human neurons using iPSCs differentiated to a neuronal fate. Further, in collaboration with the HSCI iPSC Core Facility, we generated new iPSC lines from two parties with AD-causing genetic mutations in APP. We hypothesize that disease-specific human neurons derived from iPSCs with AD-causing mutations will demonstrate either significant increases in A secretion overall or significant increases in the secretion of especially toxic A species. Our preliminary data suggest that our assay can detect differences in A secretion between iPSC lines. By characterizing A production by these neurons, we seek to investigate how familial AD mutations (and eventually how several other minor risk alleles for AD) affect A generation and secretion.

Building a Behavioral Map of Dopamine Subcircuits in Drosophila

Abstract

A holy grail of neurobiology is the elucidation of neural circuits that modulate specific behaviors. Manipulation of dopaminergic neurotransmission has been shown to affect a variety of behaviors in *Drosophila melanogaster*, but the specific neuronal circuitry underlying many of these behaviors remains unclear. To identify the subcircuits of dopaminergic neurons that are possibly involved in regulation of aggression and courtship behavior in *Drosophila*, I used a well-established TH-GAL4 driver line in combination with the Flippase (FLP)-recombination technique to restrict the extent of TH-GAL4 expression. Using the collection of enhancer-trap FLP lines (the FLP library) that represents a random survey of the transcription patterns within the genome, I searched for overlapping FLP and TH-GAL4 expression patterns that target different subgroups of dopamine-containing neurons. I then silenced these subgroups of dopaminergic neurons and performed behavioral assays to screen for any phenotypes associated with targeted restriction of dopaminergic neurotransmission. My results show that a pair of dopaminergic neurons in the subesophageal ganglion (SOG) and a pair of dopaminergic neurons in the superior posterior slope (PPM3 cluster) may inhibit aggression. Furthermore, neurons in the posterior inferiorlateral protocerebrum (PPL1) and PPM3 clusters may play a role in modulating locomotion. My work suggests that similar approaches can be used to build a behavioral map of *Drosophila* brain circuitry, facilitating future work towards discovering the neural circuits underlying behavior in higher organisms.

Categorizing Cell Types of the Mammalian Organ of Corti: deepCAGE and the Effect of Pax2-Sox2 Synergy on Atoh-1 Expression

Abstract

Over the course of evolution, mammals have lost the ability to regenerate mechano-sensory hair cells, the cells necessary for auditory transduction. Two native cell populations have been suggested as potential targets for hair cell regeneration: Organ of Corti supporting cells and endogeneous dormant stem cell spheres¹. The first part of this thesis utilizes deep Cap Analysis of Gene Expression (deepCAGE) to produce an Organ of Corti “promoterome:” a database that outlines what transcriptional start sites (TSS’s) are active in hair cells, supporting cells, and endogeneous Organ of Corti dormant stem cell spheres. Additionally, deepCAGE data provides insight into how robustly these TSS’s are being expressed. We hope that this database will provide future gene candidates for hair cell regeneration. Following deepCAGE analysis, part two of the thesis explores the roles of Pax2 and Sox2 in defining hair cell fate. We suggest that these two early transcription factors co-bind in hair cell progenitors, subsequently up-regulating Atoh-1, a transcription factor responsible for hair cell fate. We suggest that Sox2+Pax2+ progenitors will ultimately become hair cells, while Sox2+Pax2- cells become supporting cells.

Effects of Counterfactual Thinking on Source Monitoring during Memory Retrieval

Abstract

This research investigated potential effects of positive (upward) and negative (downward) valence imagined alternative outcomes to past experiences, referred to as counterfactual simulations, on episodic memory of these experiences. For forty sets of tasks, which could be performed in two different ways, participants were told that most people preferred one way of performing the task and were then asked to choose what they considered the most popular action for each pair. For trials that were determined to be downward counterfactual trials, participants were told that they made the unpopular choice and received no monetary reward. They always received monetary reward for upward counterfactual trials and had an equal chance of receiving or not receiving reward for control trials. Following a distractor task, in the subsequent imagination phase of the experiment participants engaged in upward counterfactual thinking by simulating performing and receiving a reward for tasks they did not choose in the previous session. They also carried out downward counterfactual simulations of performing unrewarded tasks they did not choose in the previous session. A week later, in an old/new recognition test that included previously performed, imagined, and novel tasks, participants identified tasks that had been physically performed during the first phase of the experiment (old) and ones that had only been imagined subsequently or were entirely novel (new). Participants displayed significantly higher false alarm rates for counterfactually simulated outcomes of tasks compared to novel tasks, with no difference between upward and downward counterfactuals. Results from older adults ($n = 6$) show a larger counterfactual thinking effect, but are still exploratory and do not show a significant age by condition interaction due to small sample size. The results indicate that imagining alternative outcomes to past events may lead people to recollect incorrectly the alternative instead of the original event.

The Art of Perception: Exploring Differences in the Aesthetic Experience of Abstract and Representational Paintings

Abstract

The research presented in this thesis was conducted in the Laboratory for Perceptual Dynamics at the RIKEN Brain Science Institute, under the direction of Dr. Cees van Leeuwen. I had the pleasure of working as a member of Dr. van Leeuwen's research team during the summer of 2009, under the mentorship of Massimiliano Palmiero. I designed and performed all experiments that are reported as results. The experiments described in Chapter 2 were performed between June and August 2009. The FFT Analysis and ANOVAs were completed between September 2008 and March 2009, with invaluable programming assistance from Chie Nakatani. The art history portion of this thesis was completed between August and January 2010. In total, this work was done over two summers and during three semesters of coursework.

This thesis is presented in partial fulfillment of the requirements for the Bachelor of Arts degree with honors in History of Art and Architecture and Neurobiology. Because my primary concentration is History of Art and Architecture, this thesis is formatted according to the guidelines provided by the History of Art and Architecture Department.

Neurobiology of Economic Decision Making: The Influence of Confidence on Delay Discounting

Abstract

Though normative economics assumes rational agents, animals often deviate from this model. Specifically when faced with intertemporal choices in which disparate alternatives are delivered at different times, organisms often compromise overall utility by selecting a more immediate reward. In this thesis, I sought to understand the role of risk aversion in driving this bias. In addition to temporal preference, the avoidance of risk may contribute to delay discounting, as delayed reward is inherently uncertain, though a previous study demonstrated that eliminating risk greatly reduces the tendency to discount future reward, the means by which uncertainty discounts future reward are unknown, Risk could plausibly affect the discounting phenomenon in one of two ways: decreasing general preference for delayed, albeit larger, reward, or alternatively, increasing the rate of discounting future reward. I hypothesized that when confidence of future reward delivery increased, the discounting rate would change, In an experimental paradigm, rats were offered immediate, smaller reward and larger, postponed reward. I assessed the animals' choices when the larger reward was associated with different time delays and also under the same conditions, but with the element of risk manipulated. As expected, the bias toward immediate reward was mitigated by reducing the risk associated with larger reward. I quantified the rates at which the animals discounted and discovered that minimizing the risk associated with delayed reward changed the time constant of the temporal discounting curve. The present result thus demonstrates that risk together with pure temporal preference (impatience) regulates the temporal discounting function.

Evaluating developmental differences in the neural correlates of executive functioning: an fMRI study

Abstract

This thesis seeks to characterize the neural correlates of inhibition and working memory in neurologically normal children ages 5-10. In order to achieve this goal five children in each age group were recruited. Each subject was given a standardized assessment of reading, math, IQ, and executive functioning in order to ensure they were typically developing, and their parents were asked to fill out questionnaires regarding the mental state and function of their child. Functional magnetic resonance imaging (fMRI) was used to characterize neural correlates; in order to evaluate inhibition, the Simon task was used, and in order to evaluate working memory, a delayed match to sample (DMS) task was used. General linear model regressions were created using fMRI data analysis tools, and behavioral responses regarding response accuracy and reaction time were collapsed across conditions for each task. Additionally, responses to standardized assessments and parent questionnaires were scored. Statistical analyses were run to evaluate the effect of age on the various assessments.

Due to low behavioral accuracy regarding both executive functions evaluated in the fMRI, neural correlates could not be identified regarding the development of inhibition and working memory. This study showed that age-related decreases in activation of the right inferior parietal cortex and medial frontal cortex were related to improvements in task proficiency in congruent and incongruent trials in inhibition. This study also showed an age-related decrease in activation of the precuneus was related to improvements in task proficiency in high load DMS trials. These age-related decreases in brain regions could be attributed to older subjects requiring less attention to visual stimuli and motor control as compared with younger subjects when performing these tasks.

Modeling neuroimmune communication after peripheral nerve injury

Abstract

Pain resulting from peripheral or central nervous system injury (neuropathic pain) is usually persistent and difficult to manage. Peripheral neuropathic pain is associated with an immune response in the dorsal horn of the spinal cord, mediated by activation of resident microglial cells and recruitment of peripheral immune cells such as macrophages. Precisely how and why these cells are recruited and activated in the spinal cord, a location remote from the actual lesion site, remains unknown. In this study, we sought to determine whether signals released from primary sensory neurons are responsible for the immune response. We focused on the role of fractalkine (Cx3cl1), because this chemokine is exclusively released from neurons and acts through a single receptor expressed on both microglial cells and macrophages. To examine the role of Cx3cl1, we used a knockout mouse, in which the fractalkine receptor gene has been deleted and replaced by green fluorescent protein. Using flow cytometry, we found that in the absence of Cx3cl1-mediated signaling, the recruitment of peripheral macrophages after peripheral nerve injury decreases. However, an increase in spinal microglial cells compensates for this loss. This compensation is functionally complete, with no resulting differences in neuropathic pain-like behavior. We designed experiments to determine what other neuroimmune signals are released upon peripheral nerve injury to mediate this compensation. Thus we developed an in vitro neuroimmune co-culture technique, which will allow for identification of molecular signals exchanged between neurons and immune cells via axonal transport or release. In future experiments, we will manipulate neuronal activity in a temporally precise manner using neuronal transfection with channelrhodopsin-2, a light-gated ion channel. By analyzing activity-dependent neuroimmune communication after nerve injury, novel therapies can be developed to prevent or reduce chronic neuropathic pain.

Neural Activation during Task Performance Predicts Practice-Based and Off-line Behavioral Improvement

Abstract

Recent research involving memory consolidation has revealed that learning involves both an active component that occurs during task performance and an off-line component during the subsequent rest period. The mechanisms that underlie these two aspects of consolidation are not yet completely understood. In our current research, we aimed to relate activation during a task to both types of memory consolidation. Using functional magnetic resonance imaging (fMRI), we examined the extent to which cortical activation differences between a finger-tapping Motor Sequence Task (MST) and a Motor Control Task (MCT) that included motor activity but not learning were able to predict practice-based and next-day improvement. We found significant practice-based and overnight behavioral improvement on the MST, indicating that learning and off-line consolidation had taken place. In terms of activation, we found increased activity in contralateral sensorimotor areas during the MST as compared to the MCT, along with bilaterally decreased activity in the primary visual cortex (V1), anterior cingulate cortex (ACC), and the orbitofrontal cortex (OFC). Activation in several of these areas, including the ACC, posterior cingulate cortex (PCC), the insular cortex, and the primary motor cortex (M1) was correlated with increased practice-based improvement but decreased overnight improvement. Although the correlation was bilateral, more significant correlations were seen in the contralateral hemisphere. These results suggest that practice-based and overnight improvement are products of two separate processes, the latter of which likely depends on resting state activity after completion of the task.

**The role of Exchange Protein Directly Activated by cAMP (Epac) in the Mst3b Pathway
Leading to Cortical Cell Axon Outgrowth and Regeneration.**

Abstract

The regenerative potential of neurons in the CNS is governed by intracellular signaling pathways, including the cAMP pathway and a novel pathway mediated by protein kinase Mst3b. It has been shown that the GTPase Rap1 is upstream of Mst3b in this pathway, but it is as yet unknown which of Rap1's various GEFs activate Rap1 for Mst3b-specific activity. The Rap1 GEF most closely linked to axon growth and regeneration is the exchange protein directly activated by cAMP [Epac]. As such, we investigated the relationship between cAMP, Epac, Mst3b and axon growth in E18 rat cortical neurons in vitro, a system where Epac's role in axon growth has not yet been defined. We activated or inhibited Epac and Mst3b pharmacologically and via shRNA transfection and measured initial axon outgrowth and axon regeneration after injury. Inhibiting PKA, the other main effector of cAMP besides Epac, did not affect axon outgrowth. On the other hand, pharmacologically inhibiting Epac strongly inhibited outgrowth, as did inhibiting Mst3b. Activating Epac did not have an effect on outgrowth, while activating Mst3b had a moderate one. Epac was shown to be a significant mediator of axon outgrowth in rat embryonic cortical cells, along with Mst3b. Although these results cannot conclusively establish Epac's hypothesized role upstream of Mst3b, they support that model.

The Role of Semaphorin 5A within the Injured Adult Peripheral Nervous System

Abstract

A genome-wide single nucleotide polymorphism association study run by the Woolf lab implicates semaphorin (Sema5A) as a candidate gene that increases the susceptibility of patients to neuropathic pain. Single Nucleotide Polymorphisms (SNPs) within and around Sema5A had an extremely high correlation with pain that persisted at least 1 year after surgery to remove herniated disc(s), which were impinging onto patient's dorsal roots. Lesions to peripheral nervous system can result in aberrant axonal growth and maladaptive synaptic strengthening, which in turn can lead to neuropathic pain. Here we investigate whether sensory neurons from dorsal root ganglion (DRG) express Sema5A and if so, whether its depletion affects the axonal growth.

Antibody staining of primary DRG cell cultures shows that Sema5A is expressed in the neuronal soma, axon growth cone and at the points at which axons split. Knock down of Sema5A protein in cultured DRG neurons using lentiviral delivery of shRNA significantly reduced neurite length by 91.4% compared with the control shRNA delivery. Depletion of Sema5A protein also dramatically reduced the number of sensory neurons with neurite initiation by 95%. In addition, the knock down of Sema5A dramatically increased beta-tubulin expression within non-neuronal cells from undetectable to readily detectable levels.

These in vitro data suggests that an alteration in Sema5A significantly influences axonal growth, as well as altering the cytoskeleton of the non-neuronal cells. Further in vivo and in vitro experiments will be needed to clarify the nature and mechanism of the role of sema5A in both neuronal regeneration and neuropathic pain.

Mental Disorders and the Basal Ganglia dysfunction: Neural Mechanisms of interval timing in behaving mice

Abstract

This study was part of a larger investigation using the mouse as an experimental model to explore the role of basal ganglia dysfunction in mental disorders. The ultimate goal of this study is to understand how impairments in basal ganglia circuit function might lead to mental disorders that involve dopaminergic pathways, which range from schizophrenia and bipolar disorder to the cognitive aspects of Huntington's disease and Parkinson's disease. Pharmacological, lesion, electrophysiological, and imaging data from both animals and humans have supported the hypothesis for the cortico-striatal representation of time and its link to mental disorders, but this study is the first attempt to investigate the neural mechanisms of timing using neurophysiological techniques in behaving animals. The larger project requires multielectrode recordings in trained, head-fixed mice as they perform a behavioral task. Therefore, the immediate goal of this study is to validate that alert, head-fixed mice can routinely and reliably perform the interval-timing task, a cognitive process that involves cortex-basal ganglia-thalamus circuit function and has been implicated in a range of mental disorders. We trained mice using standard operant conditioning techniques with positive reinforcement to depress a lever with a forepaw and release it after a specific interval of time had elapsed. The results show that alert, head-fixed mice can routinely and reliably perform 500-1000 trials during daily 1-hour sessions, which will suffice to enable pending experiments to use neurophysiological techniques to delineate the neural mechanisms of the interval-timing task at each stage of the cortex-basal ganglia-thalamus circuit and investigate the effects of activating the direct and indirect pathways of the basal ganglia on behavioral and neuronal responses.