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THE NEUROSCIENCES INSTITUTE

SCIENTIFIC REPORT

December 2005

Table of Contents

DIRECTOR'S INTRODUCTION

The ultimate goal of our work is to understand the human brain in health and disease.

Scientists at The Neurosciences Institute seek to understand how the brain works. Their research ranges over a number of different animal species and applies sophisticated experimental techniques at all levels of brain organization. At the same time, their efforts rely on theoretical approaches designed to understand the complexity of the brain. As in previous reports, the reader of the present report will see all of these approaches in full use.

To achieve its goal, The Neurosciences Institute is deliberately kept small: its scientific personnel never exceeds 40 researchers. This allows rapid exchange and cooperation among the researchers regardless of specialty. Scientists here are rewarded for their ability to sharpen questions and solve problems, rather than for any of their specialist skills. They share the belief that global brain theories and neural modeling in powerful computers are essential to interpret the diversity of their experimental results. Accordingly, there is particularly close communication between theorists and experimentalists.

By applying all of these organizational principles, progress has been made across a variety of fronts. These range widely from studies of learning, perception, and sleep in rodents and fruit flies to studies in humans of higher order brain responses to rhythm and melody. At the same time, we have made advances in the understanding of synaptic mechanisms, of gene expression in the brain, and of motor learning. Recognizing the importance of theoretical efforts to consolidate these areas of study, researchers here have designed and built a number of brain-based devices. Like animals and unlike computers, these devices are not programmed. Instead they learn from their experience. Guided by their brain structure and dynamics, they can correct mistakes in terms of that experience.

The ultimate goal of our work is to understand the human brain in health and disease. The results of our efforts have been reported in a number of respected scientific journals. We remain dedicated, as well, to communicating the insights we have gained to the public, especially the local community.

Gerald M. Edelman, M.D., Ph.D.
Director

THE NEUROSCIENCES INSTITUTE: HISTORY

The Neurosciences Research Program (NRP) was founded in 1962 at the Massachusetts Institute of Technology. In the recognition that traditional barriers between disciplines had to be removed if brain functions were ever to be understood, the NRP developed innovative formats for intellectual exchange among scientists from diverse disciplines along with effective programs for disseminating knowledge about brain science within the broader scientific community.

After a degree of success in its activities over two decades, the NRP leaders recognized the need for a different kind of scientific approach—one that emphasized formulation of scientific questions for future research, rather than only the assessment and dissemination of current knowledge. To this end, The Neurosciences Institute was established in 1981 and located as an independent entity on the campus of The Rockefeller University in New York City. The NRP moved its operations to the Rockefeller in 1983.

The Institute began by sponsoring various activities for visiting scientists; these programs were generally organized at the suggestion of individual scientists around a focused research problem. Over the last two decades, more than 1,100 scientists from 300 institutions and 24 countries have visited the Institute to meet informally in small groups to exchange information, to plan experiments to be carried out upon return to their home institutions, or to prepare critical evaluations of current research for communication to the scientific community.

In 1988, the Institute began its own program of research in theoretical neurobiology. Carried out by a group of specially appointed resident Fellows, the program was designed to develop biologically based theories of higher brain functions and to train young scientists in the methods used to construct such neural theories.

In 1993, the Institute moved from New York to temporary quarters in La Jolla, California, while permanent facilities were being constructed nearby on land owned by The Scripps Research Institute. The new three-acre campus on Torrey Pines Mesa was officially opened on October 15, 1995. The three-building complex, designed by the architecture firm Tod Williams Billie Tsien and Associates, has been awarded numerous honors, including the Honor Award for Architecture from the American Institute of Architects in 1997.

The new quarters included experimental laboratories for research across a broad front of neurobiological disciplines, as well as facilities for theoretical research and for visiting scientists. With the laboratories in place, a research program for Fellows in Experimental Neurobiology was begun, thus fulfilling the original plan for the Institute's full range of scientific activities.

The complex also includes an auditorium, designed by Williams and Tsien in consultation with the renowned acoustician Cyril Harris, that seats 352 persons. It is used regularly for scientific presentations. To take advantage of its superb acoustics and to provide a resource for the community, the Institute allows local performing arts and non-profit groups to use the auditorium without charge under the "Performing Arts at The Neurosciences Institute" rubric.

The Institute continues to be the home of the NRP, known internationally as a very prestigious small academy. The NRP consists of a maximum of 36 members elected from among the most greatly accomplished neuroscientists and other scientists interested in brain function. It meets yearly at the Institute, and its members serve in the interim to offer advice that might be necessary within various neuroscientific specialties.

The Institute operates under the aegis of the Neurosciences Research Foundation, Inc. (NRF), a publicly supported, tax-exempt, not-for-profit organization. Its Board of Trustees includes representatives of the general public as well as scientists. The Institute is supported largely by gifts and grants to the Foundation from non-governmental sources, including individuals, foundations, and corporations. Certain projects also receive governmental support. The flexibility that these modes of support engender has proven to be important for the organizational and operational styles of the Institute. NRF is deeply grateful to all of its donors, who are acknowledged specifically in the separate *BrainMatters* annual report publication.

SCIENTIFIC REPORT

The following report summarizes important salients in the research carried out by Fellows at The Neurosciences Institute in the period ending December 31, 2005. It includes new work in the theoretical and the experimental neurobiology programs carried out since that described in the previous edition of the *Scientific Report,* which covered the period through June 30, 2004. The current research staff and former Fellows are listed below. The summaries that follow describe the scientific questions being pursued, the accomplishments to date, and the importance and potential practical applications of the new knowledge. Each summary includes the names of the principal researchers involved along with selected references to the list of publications at the end of this section. More detailed descriptions of the research, as well as of related projects not described in these summaries, may be obtained from these publications. Additional information about the Institute's various activities can also be obtained from the Institute's web site: www.nsi.edu.

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Research in Theoretical Neurobiology

Brain-Based Devices

Over the last 14 years, The Neurosciences Institute has successfully constructed brain-based devices (BBDs) to test theories of the nervous system having to do with perceptual categorization, primary and secondary conditioning, visual binding, motor control, and memory. BBDs can be classified as belonging to a class of neurorobotic devices based on features of vertebrate neuroanatomy and neurophysiology, emphasizing an organism's interaction with the environment, and strictly constrained by basic design principles. These include: (1) The device is situated in a physical environment. (2) The device engages in a behavioral task. (3) The device's behavior is controlled by a simulated nervous system having a design that reflects the brain's architecture and dynamics. (4) It's behavior is modified by a reward or value system that signals the salience of environmental cues and modulates the BBD's nervous system. Although the principal focus of developing BBDs has been to test theories of brain function, devices constructed according to neurobiological principles may also provide bases for practical applications.

Spatial and episodic memory in a brain-based device

Autobiographical memory or episodic memory requires putting together the "what," "when," and "where" of events in one's life. The storage and retrieval of such memories requires an area of the brain called the hippocampus. The BBD Darwin X incorporates aspects of the detailed anatomy and physiology of the hippocampus and surrounding brain regions (cortical areas for vision, space, and self-movement) known to be necessary for the acquisition and recall of spatial and episodic memories. Darwin X successfully demonstrated the acquisition and recall of both spatial and episodic memories in a maze task (i.e., it finds a hidden location on the floor of a large room by associating places with actions). After a period of autonomous exploration, Darwin X could remember the hidden location and would move directly toward it from any starting place in the room. Successful navigation by Darwin X required it to localize its position through local and global cues, learn appropriate actions in a given context, and develop goal-directed behavior. All of these attributes are applicable to navigation in natural settings, an area which The Neurosciences Institute is actively pursuing.

By putting together the "what," "when," and "where" of events, as well as integrating multimodal information over temporal sequences, Darwin X demonstrated both episodic memory and spatial memory. The detailed hippocampal anatomy incorporated into Darwin X facilitated the integration of inputs over different timescales. The responses of simulated neuronal units in the hippocampal areas during its exploratory behavior were comparable to those of neurons in the rodent hippocampus; i.e., neuronal units responded to a particular location within Darwin X's environment. Moreover, like the rodent, these "place cells" were context-dependent; their responses were correlated both with where the device had been and where the device was going.

Darwin X is the first BBD model that takes into consideration the macro- and micro-anatomical connections between the hippocampus and cortex, as well as those within the hippocampus. In order to identify different functional hippocampal pathways and their influence on behavior, two novel methods for analyzing large-scale neuronal networks were developed at The Neurosciences Institute: 1) "Backtrace," which involves tracing functional pathways by choosing a unit at a specific time and recursively examining all neuronal units that led to the observed activity in this reference unit, and 2) "Causality," a time series analysis that distinguishes causal interactions within and among neural regions. These analyses allowed us to examine the information flow through the network and showed a relationship between experience and network integration; more stages of hippocampal processing were required when in novel environments than in familiar ones. This work has provided new approaches to understanding the complexity of network dynamics, and the methods of analysis have applicability to real nervous systems and networks in general.

Fine motor control in a model of the cerebellum and cortical visual motion areas

The cerebellum is an area of the brain known to be critical for accurate motor control. A recent theory of cerebellar function proposes that the cerebellum learns to replace reflexive movements with a predictive signal that produces the correct movement before an awkward reflex occurs. A BBD was constructed which incorporated a detailed model of the cerebellum and cortical areas that respond to visual motion. We tested

whether a novel learning mechanism, called "the delayed trace learning rule," could account for the predictive nature of the cerebellum in a robotic, real-world visuomotor task. The platform for this BBD was based on a modification of the Segway Human Transporter balancing technology, called the Robotic Mobility Platform (RMP). The BBD received input from a video camera and infrared proximity sensors, and the cerebellar model sent motor output to control the wheels of the RMP.

The BBD's visuomotor task was to navigate a path defined by rows of orange traffic cones. Initially, navigation relied on a reflex from infrared proximity sensors that were triggered when the BBD was within 12 inches of a cone; this resulted in clumsy, crooked movement down the path. The infrared sensor input, which drove the reflex, was also used as an error signal to the cerebellum. Over time, the cerebellar circuit became able to predict the correct motor response based on visual motion cues through its interaction with the visual cortical areas. This prevented the activation of the reflex and resulted in smooth movement down the center of the path. After training, the system was tested using only visual cues, and it was able to traverse the course without error. These tests were conducted with gentle curves and then with sharp (almost 90 degree) turns. The system learned to slow down prior to a curve and to turn in the correct direction based on the flow of visual information. These experiments demonstrated how the cerebellum can predict impending errors and adapt its movements. In addition to understanding how fine motor control is established in humans, the model may have significant practical applications for machine control.

A soccer-playing brain-based device

We have applied the visual binding and scene segmentation model of the BBD called Darwin VIII, as well as neural models for motor control and action selection, to a new BBD that can play soccer in both indoor and outdoor environments under varying lighting conditions and surfaces. The fully autonomous soccer-playing device was built on the Segway RMP. It demonstrated all aspects of soccer play (shooting, passing, catching, maneuvering towards goals, etc.). Kicking to a teammate or to the goal was achieved by the device's recognizing the appropriate object, centering the object with its camera field of view, and then kicking the ball. An action selection system enabled the BBD to autonomously choose plays (e.g., run downfield, defend a goal) or behaviors (e.g., pass, shoot, catch) based on the position of field objects (e.g., teammate, opponent, goal) and the game context (e.g., ball possession, on offense). A route planning system allowed the BBD to head towards goal objects or locations while avoiding obstacles. It integrated information from the visual system, which recognized objects on the playing field (e.g., balls, goals, players), with laser rangefinder readings to obtain the distances to the objects.

The rules of Segway Soccer are a combination of those of soccer and Ultimate Frisbee. A team consists of a human, riding a Segway Human Transporter, and an autonomous Segway RMP. Players with possession of the ball must either pass to a teammate or shoot at the goal; neither human nor robot player can advance while possessing the ball. The soccer playing capabilities of the BBD were demonstrated in a series of matches against a team from Carnegie Mellon University who developed a robotic Segway RMP. The competition was held at the RoboCup US Open 2005 in Atlanta, Georgia. The scores of the games were 3-0, 4-0, 3-0, 3-0, and 2-1, all in The Neurosciences Institute's favor; the human players were not allowed to shoot for goals in the last two games. Video clips of the games can be found at http://www.nsi.edu/nomad/segway. The Segway Soccer BBD demonstrated some of the advantages of the brain-based approach in dynamic, complex environments and in tasks that involve interaction with humans. (Gerald Edelman, Jason Fleischer, Donald Hutson, Jeffrey Krichmar, Jeffrey McKinstry, Douglas Moore, Anil Seth, James Snook, Botond Szatmáry; see publications 512, 518, 541, 546, 551, 553, 554, 561, 566, 576)

Theoretical Neuroanatomy and Neural Dynamics

The Neurosciences Institute has long been at the forefront of the field of theoretical neuroanatomy, which reflects the study of network structure and network dynamics as they relate to neuroscience. Previously, this research has shown that "complex" neural dynamics accompany adaptation to rich sensory and motor demands, where complexity in this context is defined as a balance between dynamical segregation and dynamical integration. This work has been extended by adapting techniques from

time-series econometrics to characterize causal interactions in neuronal networks, providing new tools to aid in understanding the complexity of network dynamics in terms of the density of causal interactions.

Recently, a novel causality analysis has been applied to the dynamic neural activity generated in the BBD Darwin X (see above), permitting a rigorous discrimination of causal interactions from contextual interactions. The analysis generated several hypotheses regarding the functional roles of known anatomical pathways through the hippocampus. Our research into causality has implications for the design of flexible and robust neuronal network control systems, and it provides new methods for analyzing neural dynamics generated by biological as well as simulated systems.

Analyses of the dynamics of human brain activity using magnetoencephalography (MEG) have focused on the potential for detection of deception. The project involves a paradigm in which subjects were encouraged to lie spontaneously in order to gain modest financial rewards. By analyzing MEG signals in the alpha frequency range (8-12 Hz), it was possible to predict deceptive responses on a trial-by-trial basis with accuracies approaching 80%. Moreover, MEG signals had a much higher predictive accuracy than the "skin conductance" signals typically used in lie detection. In a different paradigm, deceptive responses were found to correlate with a reduction in the coherence among certain MEG sensors. This research has implications not only for the ability to detect deception, but also for the use of brain signals to predict the occurrence of cognitive states in general. MEG is currently being applied to explore the basic cognitive mechanisms involved in deception, such as working memory and risk management, as well as neural dynamics during the transition from sleeping to waking. (Anil Seth, Jeffrey Krichmar, John Iversen, Ani Patel; see publications 510, 529, 561, 576, 585)

Models of Learning and Reward

Learning the associations among cues, actions, and rewards involves selective reinforcement of neuronal activity. Typically, the reward comes some seconds after reward-predicting cues or reward-triggering actions. This creates an explanatory conundrum known in the behavioral literature as the "distant reward problem" and in the reinforcement learning literature as the "credit assignment problem." How does an animal know which of the many cues and actions preceding the reward should be credited for the reward? In neural terms, when sensory cues and motor actions correspond to neuronal firings, how does the brain know what firing patterns are responsible for the reward if the patterns are no longer there when the reward arrives? How does it know which neuronal spikes result in the reward if many neurons fire during the intervening period? Finally, how does the common reinforcement signal in the form of the neuromodulator dopamine (or a similar neuromodulator) get to the right synapses at the right time, assuming dopamine is released globally to many synapses?

We have shown how the credit assignment problem can be theoretically solved in a network of cortical spiking neurons with spike-timing-dependent plasticity (STDP). If it is hypothesized that STDP is modulated by dopamine in the same fashion as it affects long-term potentiation and long-term depression, it was demonstrated that dopamine-modulated STDP has a built-in property of instrumental conditioning: it can reinforce firing patterns occurring on a millisecond time scale even when they are followed by rewards delayed by seconds. We have made a biologically plausible spiking network model for reinforcement learning using dopamine-modulated STDP. It illustrates how reward-triggered release of dopamine shifts from the time of the unconditioned stimulus to that of reward-predicting conditioned stimulus. (Eugene Izhikevich, Jeffrey Krichmar, Jeffrey McKinstry)

Models of a Single Neuron

Analyses of the dynamics of living neurons and their detailed simulations have allowed the formulation of a new computational model of neuronal dynamics that combines the biological plausibility of earlier Hodgkin-Huxley-type models with the computational efficiency of integrate-and-fire models. In particular, this new model can generate firing patterns of all known cortical, thalamic, and hippocampal neurons, as well as mitral cells in olfactory bulb, spiny projection cells of the neostriatum, and stellate cells of entorhinal cortex. The parameters of this model have been fine-tuned so that the simulated voltage responses are practically indistinguishable from those that have been experimentally recorded in various research laboratories. The equations underlying this model provide a

foundation for the development of BBDs governed by a simulated nervous system composed of spiking neurons. (Eugene Izhikevich; see publications 528, 533)

Large-scale Brain Simulations

The Institute has a long-standing tradition of constructing anatomically detailed models of the brain, focused particularly on the thalamocortical system. Using the latest anatomical data on the synaptic microcircuitry of cat visual cortex, a large-scale thalamocortical model of unprecedented detail has been developed. This model contains six-layered cortical anatomy with 24 different types of multi-compartmental spiking neurons. It contains specific, nonspecific, and reticular nuclei of the thalamus. The neurons have receptors with AMPA, NMDA, GABAA, and GABAB kinetics, show STDP, show short-term synaptic depression and facilitation, and exhibit axonal conduction delays. The STDP and the delays play important roles in shaping the fine temporal structure of neuronal firing and lead to the emergence of neuronal groups.

A simpler version of the model (the same anatomy but no plasticity) was recently scaled up to the size of the human brain–10^11 neurons and 10^15 synapses corresponding to 30 cm x 30 cm of cortical surface. The simulation of one second of neural activity in the model took 50 days on a Beowulf cluster of 27 processors. This is the largest simulation of an anatomically detailed neuronal model that has ever been carried out. It provides a benchmark for the resources needed to simulate models with the size of the human brain. (Eugene Izhikevich, Joseph Gally, Gerald Edelman; see publication 584)

Neural Reapportionment

Although sleep is a ubiquitous feature of animal life, and prolonged sleep deprivation is fatal in both vertebrates and invertebrates, the physiologic function of sleep is not known. In our theoretical work we proposed that sleep provides a period of time necessary to reapportion resources within neurons and neural systems that become sub-optimally distributed during active waking. Three specific examples of such reapportionment during sleep were suggested: (1) the return of the neurotransmitter, glutamate, to synaptic vesicles at presynaptic sites most active during waking, (2) the intracellular movement of mitochondria from neuronal processes to the cell soma where mitochondrial replication can occur, and (3) the readjustment of the level and distribution of neurotransmitters within the brainstem modulatory systems and elsewhere that must function in an integrated fashion during waking. Experimental approaches that might be utilized to test these hypotheses were suggested. (Joseph Gally, Gerald Edelman; see publication 522)

Music and Brain Function

Music is attractive as a subject of research in neuroscience because it engages many fundamental aspects of brain function and is deeply meaningful to humans, yet is constructed from basic elements which can be easily manipulated and measured for scientific purposes. One major line of research at the Institute has been the study of music's relationship to language. We continue to find important links between the two domains in terms of structure and cognitive processing. These links help illuminate underlying brain processes involved in both domains and challenge the traditional view that music is a neurally isolated brain function. A second important line of research has used music (or stimuli inspired by music) to address basic issues concerned with how the human brain perceives patterns in time. Using magnetoencephalography (MEG), we have explored the mechanisms of selective auditory attention, the perceptual separation of sound sources by the nervous system, and the interaction of stimulus-driven vs. internal cues in the brain's processing of rhythmic patterns.

Comparing rhythm and melody in speech and music

Humans produce organized rhythmic and melodic patterns in two forms: prosody and music. Yet these patterns are typically studied by different research communities, and there has been remarkably little empirical work comparing rhythmic or melodic structure across domains. Such work is warranted for the broader perspective it yields on how the mind processes structured temporal patterns. In previous research, we showed that the rhythm of a composer's native language is reflected in their instrumental music, providing the first scientific evidence for an intuition that had been voiced by scholars of music and language for over half a century. In more recent work we have extended our research to melody. Using recent research on the perception of speech

intonation, we have quantified statistical patterns in the speech melody of two languages (British English and French), using methods which can also be applied to the study of pitch patterns in instrumental music. We find that empirical differences between English and French speech intonation are reflected in the melodic patterns of instrumental classical music in the two cultures. Combining this finding with our earlier work on rhythm, we have developed a novel way to represent the position of a nation's language (or its music) in a two-dimensional space with rhythm and melody on orthogonal axes, permitting measurement of the "prosodic distance" between languages or musics. The cognitive significance of this work is that it suggests that implicit learning of patterns in one domain (speech) can influence the creation of patterns in another domain (music), showing that the mind does not enforce a strict division between the learning of music and language.

Language and music: What are the common processing mechanisms?

Work on the relation between the perception of music and language has taken several forms. One question is perceptual, asking if expectations about sound learned in one domain influence perception in another. It is well known that perceptual categories, e.g., for phonemes, learned for one's native language can have a strong influence on perception (or more usually, misperception) of other languages. We have shown additionally that one's native language can have a profound effect on how one perceives even non-linguistic sounds. In a large study population, we found that native speakers of English and Japanese perceive a simple rhythm of alternating long and short notes in very different ways: English speakers hear it as composed of repeating groups of short-long notes, while Japanese speakers hear it in the opposite way, as groups of long-short notes. This finding was surprising, and exciting, because it has been thought for over a century that perception of simple rhythms followed innate rules. Our research shows instead that it can be affected by experience.

One natural possibility for explaining the perceptual difference is language itself. It is well known that different languages have different rhythms (imagine the difference between, for instance, German and Italian). Perhaps extended experience hearing a language has a fundamental impact on basic perceptual processes. To investigate this possibility, we studied the rhythm of frequent words in Japanese and English. We found that the rhythm of two-syllable words was highly consistent with the perceptual biases: English has many words with a short-long rhythm (a short first syllable followed by a longer second syllable, e.g., "because" or "about") while Japanese two-syllable words tend to be the opposite, with a long syllable followed by a short one. Other rhythmic differences between the languages are similarly coherent with the perceptual biases. This suggests that experience with a language could account for the perceptual differences we observed.

Syntax in music and language

Like language, music is a human universal involving perceptually discrete elements organized into hierarchically structured sequences in principled ways. In other words, music is syntactic. Comparative research on music and language can thus be used to explore the brain mechanisms underlying syntactic processing, one of the most distinctive features of human cognition. Our most recent research on this issue has focused on individuals with Broca's aphasia, a disorder of language often accompanied by an impairment in the ability to use linguistic syntax to comprehend sentences. The musical syntactic abilities of aphasics have been virtually unexplored. This is possibly because cognitive science has focused on a few cases of dissociation between music and language in famous musicians who became aphasic after a stroke, but who continued to compose or play music. However, such cases may not be representative of music-language relations in the general population. Structural neuroimaging has revealed that the brains of professional musicians differ from non-musicians in a number of ways, probably as a result of neural plasticity associated with a lifetime of intensive musical training.

We have chosen to examine non-musician Broca's aphasics using standardized tests of linguistic and musical syntactic comprehension. Our initial study indicated that these aphasics did in fact show a musical syntactic deficit. This deficit could not be accounted for as a generalized consequence of brain damage or of low-level auditory processing problems. More recently, in a new study we have found that an aphasic's degree of difficulty with musical syntax serves as a predictor of their degree of linguistic syntactic deficit. This provides further evidence that

musical and linguistic syntax rely on common brain mechanisms, and it suggests that brain areas once thought to be purely "language areas" are in fact doing more general operations which can apply to structured sequences in multiple domains. Future comparative work will test hypotheses about the precise nature of these operations.

Musical tone-deafness

Musical tone-deafness refers to severe and lifelong difficulties with music perception despite normal cognitive functioning in other areas. It is thought to affect about 4% of the population. It has been claimed that tone-deaf individuals have normal perception of speech intonation (the "melody of speech"), but our previous research showed that these individuals do show deficits in intonation perception if all other cues for sentence discrimination are removed. Synthesizing these findings with psychophysical research on tone deafness and neurophysiological work on pitch perception, we have recently proposed the "Melodic Contour Deafness Hypothesis." This hypothesis suggests that musically tone-deaf individuals have an equivalent deficit in discriminating the direction of pitch change (up vs. down) in both music and speech, due to abnormalities in right auditory cortex. Under normal circumstances, speech perception is largely robust to this deficit while music perception is not. The Melodic Contour Deafness Hypothesis thus suggests that speech and music draw on common circuitry for tracking the ups and downs of pitch patterns, but that a behavioral dissociation emerges because of the different demands that speech and music put on this ability. The cognitive significance of this hypothesis is that it suggests that a behavioral dissociation does not necessarily imply a neural dissociation: the study of behavior must be distinguished from the study of neural mechanisms. Future empirical work will test this hypothesis.

Human neurophysiology of auditory perception

A major research direction concerns studying the brain as it is involved in perceiving complex stimuli. An overarching theme is the examination of the constructive nature of perception, asking how external and internal influences combine to modulate brain responses and perception. We aim to understand the mechanisms that allow us, for example, to pay attention to one stimulus over another, to focus our hearing on one instrument or melody line in a complex symphony, or to impose rhythmic structure on sound. To study such questions, we have developed techniques to dynamically measure neural responses to multiple simultaneous stimuli, using magnetoencephalography (MEG) and amplitude modulation ("frequency tagging") of stimuli. Below, we discuss three projects examining 1) attention, 2) auditory scene analysis, and 3) rhythmic hearing.

Attention

We have examined how the brain responds when paying attention to a sound versus ignoring it. We sought to determine to what extent selective attention is mediated by local changes in cortical neural activity, versus changes in the relation of activity between distant cortical regions. We measured the auditory steady-state response (aSSR) using whole-head MEG in human subjects involved in a bimodal selective attention task. The aSSR evoked by a continuous pure tone amplitude modulated at 40 Hz was recorded in trials lasting 100 seconds in which subjects were instructed to attend either to the auditory stimulus (4 trials) or a concurrent visual stimulus (8 trials). Subjects were required to detect small transient changes in the attended modality. Two measures described the aSSR: power at each sensor, reflecting local activity in cortex, and phase coherence between distant pairs of sensors (more than 12 cm apart), reflecting correlated activity in different cortical regions. For power, only three of 11 subjects had a significant difference with attention, with a relatively small effect size of 20%. For phase coherence, attention resulted in an increase in the number of sensor pairs with highly significant long-distance phase coherence in 10 of 11 subjects, with a mean of 9 times more coherent sensor pairs. The majority of coherent sensor pairs spanned the two hemispheres. These results demonstrate that local and long-distance aspects of the aSSR are affected differently by selective auditory attention. Local activity (as reflected by power) is not strongly affected, while long-distance relations (as reflected by phase coherence) are increased, particularly between sensors over opposite hemispheres. This is consistent with an increase in inter-hemispheric integration during auditory attention, and the change is similar to changes in brain responses observed in previous studies of reentry at the Institute when a visual

stimulus becomes consciously perceived or when a melody is perceived.

Auditory scene analysis

Auditory scene analysis is the process by which we analyze the complex mixture of sounds reaching our ears into individual objects. We make use of this ability every day, to hear voices in a crowd for example, but it is a notoriously hard problem for machines to solve. This ability is often lost as a consequence of age-related hearing loss, and it is not restored by hearing aids, leading many with hearing loss to complain of difficulty hearing in noisy, complex environments. We hope that investigating the neural mechanisms by which the brain accomplishes this process will aid these problems.

Our approach uses a simple stimulus that can be heard as either one or two separate auditory "objects." This gives us an approach to studying how the brain organizes perception, because we can track subjects' perception while they listen to the sound and we simultaneously record their brain responses. Because only the perception, and not the stimulus, changes, any modifications in brain response can be linked to perceptual mechanisms. In addition, using frequency tagging, we have developed an original technique to examine brain responses to individual tones within the stimulus sequence. This is not possible with traditional methods of electrophysiology and with functional imaging because of their slow time resolution. We found, contrary to expectations based on past research, that we are able to follow responses to individual tones with very high temporal precision.

Our specific approach addressed the question of how brain responses are jointly determined by the physical stimulus and by our mode of perceiving. It involves examining the auditory system during bistable auditory stream segregation, in which different perceptual interpretations can be applied to the same physical stimulus. When a sequence of three tones of high-low-high (HLH) frequency followed by a gap is presented repeatedly, the percept alternates between a grouped HLH "gallop" rhythm and perception of two independent streams, separated by frequency. To examine the contribution of stimulus and perception to brain responses, we measured the evoked response (aSSR) using MEG while subjects listened to this HLH stimulus and continuously indicated their perception as "grouped" or "streaming." The high and low tones were marked by different rates of amplitude modulation ("frequency tags"), enabling the response to each tone of the sequence to be studied with high temporal precision. The evoked aSSR followed the modulation envelope of the stimulus with remarkable cycle-by-cycle fidelity, with the instantaneous frequency of the aSSR tracking the change in amplitude modulation rate between the high and low tones. Perhaps because of the close tracking of the physical stimulus, simple single-channel measures of the amplitude and phase of average aSSRs do not appear to reflect the perceptual organization of the stimulus. We will examine whether higher-order relationships between sensor channels might reflect perceptual organization, as it is primarily these aspects of the aSSR that we have found to be modulated by attention.

Rhythmic hearing

The perceptual experience of a simple rhythm depends upon its metrical interpretation: where one hears the downbeat. This decision on where to place the downbeat, while often supported and implied by the music, is in fact subject to our control. Our research question is: How is this kind of active metrical interpretation reflected in brain activity?

We have begun to address this question using methods similar to those we used in the auditory scene analysis project: using a constant physical stimulus and manipulating how listeners perceive it. We are measuring brain responses using MEG as listeners are presented with a repeating three-beat rhythmic phrase consisting of two tones followed by a rest. In separate trials, listeners were instructed to internally place the downbeat on either the first or the second tone, yielding two metrical interpretations of the same sound sequence. As the stimulus was invariant, differences in brain activity between the two conditions should relate to metrical interpretation. Specifically, we asked if the response to a tone is modulated according to whether it serves as the downbeat in the rhythmic pattern. We examined evoked responses in several frequency ranges, including beta and gamma. Metrical organization reliably influenced responses in the upper beta range (20-30 Hz), which were larger when a tone was the downbeat in 7 of 10 listeners. Evoked responses in the gamma range (40-60 Hz) showed less consistent effects, with large differences between individuals. The beta responses appear to originate in auditory

cortex, suggesting that imagining the downbeat actually modifies brain responses to sound at an early stage, perhaps even changing perception. A second interpretation, based on the known role of beta frequencies in motor processing and long-distance coupling in the brain, is that the motor system is in fact an essential component of this purely perceptual act—an hypothesis that has wide ranging implications for the integration of perception and action. We are currently continuing these experiments with the specific aim of testing these ideas.

Extracting the beat

Most humans are able, spontaneously and with ease, to extract a regular beat from even complex rhythmic auditory stimuli, that is, to tap along with the beat of music. As far as we know, this ability is unique to humans. The mechanisms are at present poorly understood, but this ability must involve the flexible interplay of auditory and motor systems, and so the study of rhythm perception and production can provide a window into sensorimotor integration in the brain. In a study of beat synchronization, we showed that complex, strongly metrical rhythms surprisingly did not improve tapping accuracy, while complex weakly metrical (syncopated) stimuli in many cases made accuracy worse. An additional finding was that our ability to synchronize with a visual, as opposed to auditory, beat is markedly worse, suggesting that there is a privileged relationship between auditory and motor systems. (Ani Patel, John Iversen; see publications 511, 519, 520, 521, 532, 542, 565, 575, 589, 593)

Research in Experimental Neurobiology

Systems Neurobiology: Spatial Navigation

Integrating our prior experience, current conditions, and future expectations in the service of making intelligent decisions is an extremely complex process. When functioning efficiently, interactions between multiple brain regions allow an organism to be cognizant, or "aware," of the most effective action to take given the current context.

The program in systems neuroscience at The Neurosciences Institute is dedicated to determining how information regarding the past, present, and expected future is organized in the brain and how such information is actually used to select a specific course of action. In doing so, we find that particular brain regions integrate the same types of information in different ways, much as people interpret the meaning of abstract art in their own individual ways. In addition, we find that brain mechanisms which function to connect current conditions to the generation of appropriate behavioral responses are not "hard-wired," but instead are sensitive to processes of attention and learning.

At any given point in time, there are many possible courses of action from which to choose. In choosing, it is often helpful to know what the outcome of a particular course of action will be, and it is also helpful to consider some questions from multiple perspectives. Our recent research demonstrated that two brain regions, the hippocampus and parietal cortex, allow rats to consider their position in the world from two different perspectives. Neural output from each of these regions converges upon the prefrontal cortex, where the two perspectives are integrated with expected outcomes to generate behavioral decisions.

A human or an animal attempting to navigate through space to reach a goal must be aware of the site from which he sets out on a journey, how that site relates to where he would like to go, what ways are possible to reach that site, and what to expect upon arrival at the site. A fundamental problem in neuroscience is to determine how different forms of information pertinent to the production of such organized behavior are compiled. The search for the answers to this problem has focused on brain regions termed "associative" because they receive convergent input from many other brain regions. As such, they have the potential to integrate information.

Our work has taken advantage of the problem that navigation poses for any animal by challenging one of nature's master navigators, the rat, on a number of different tasks requiring efficient traversal through space. By examining the activity of individual neurons in different brain regions of rats as they attempt to solve navigational problems, it is now becoming clear how the mammalian brain subdivides complex problems. The emerging picture is one that implicates three different brain regions in the integration of information pertinent to navigational ability. These are the parietal cortex, the prefrontal cortex, and the hippocampus.

Somewhat to our surprise, these three regions were found to integrate the same types of information, but in strikingly different ways. In our studies, we examined brain responses to variations in information pertaining to the animal's position in the environment, the direction of its movement, the required locomotor behaviors (such as turns or continuous straight motion), and expected rewards at goal sites. Below, the ways in which these regions organize such information into distinct activity patterns are detailed.

In the hippocampal region, individual neurons became active in single, discrete locations on a maze. In addition to registering where in the environment the animal was, it was also clear that how the animal got there and which way it was going was tracked in the activity of hippocampal neurons. Individual neurons would, for instance, become active in a particular place only if the animal was facing a particular direction, or only if it had come to that place by making a right as opposed to a left turn, or only if it was subsequently going to make a right or left turn. In this way, the hippocampus registers not only where the animal is at present, but also where it has been and where it will go. These aspects of hippocampal activity patterns are likely related to the role of the hippocampus in "episodic memory" where the sequence in which events occur is remembered.

Whereas hippocampal neurons register where the animal is, parietal neurons register the animal's presence in a particular segment of a path. If, for instance, the animal needed to run straight, and then make a right turn, two left turns, and two right turns to reach a goal site, an individual parietal neuron might fire throughout the space between the second left and the third right turn. Remarkably, if the maze was turned 180 degrees or was doubled in size, the same neurons would fire across the same segments relative to the start and end points of the path. Thus, unlike hippocampal neurons, parietal neuron activity is not dependent on position in the room, but instead reflects position in a path, no matter where in the room that path is followed.

Finally, we found that the prefrontal cortex integrated multiple forms of information to produce yet another type of activity pattern. Here, as in the parietal cortex and hippocampus, neurons had activity increases only in particular places. The activity in such places was, however, highly sensitive to the amount of reward expected at the end of the path. That is, the prefrontal cortex appears to register the reason, or the why, of the series of path-running behaviors. One can determine from the pattern of activity of individual neurons whether the animal "thinks" it will receive a large, small, or no reward once it reaches the goal site. Remarkably, a second feature of prefrontal neuron activity was indicative of how sure the animal was that it was on the correct path. When the animal was sure, it rarely made changes in its trajectory. Under these conditions, the patterns of activity seen along the path had "high fidelity." That is, every time the animal went down the path, the activity was almost exactly the same and the animal's behavior suggested a high level of decisiveness. In contrast, when the animal took paths leading to small rewards, activity was more variable and the animal was more likely to change its mind. Interestingly, this brain area in humans is widely thought to be critical for reasoning, planning, and decision-making.

Together, the results suggest that brain regions which put together multiple forms of information do so in very different ways. An important direction for future research is to examine how these three brain regions compete or cooperate in the production of actual motor behaviors. Ultimately, the different forms of integration achieved by these areas must be put to use in deciding what behavior to perform at any given moment. Determining how this happens will enable us to "close the loop" in understanding how complex decisions are made.

Nuclei in the hippocampal formation

Neurons within nuclei of the hippocampal formation are known to be activated by specific aspects of a route traversal, such as the spatial location of the animal, specific landmarks, or the directional heading of the animal to name a few. Consequently, it is commonly thought that this area is critical in spatial learning and navigation. While certain nuclei within the hippocampal formation, such as area CA1, have been studied exhaustively on this topic, other areas, such as the subiculum and dentate gyrus, have been relatively ignored. The goal of our current research effort is to formulate a more comprehensive view of how the nuclei within the hippocampal formation individually contribute to navigation and spatial awareness.

A navigational task has been developed wherein an animal must traverse from a start location to a target location. Near the start of the traversal, the animal encounters a tactile landmark that is associated with a future intersection where a choice must be made among three directions: straight, left, or right. If the animal chooses the path paired with the landmark, a food reward is given at the target location. Thus, the animal can freely traverse to the target via multiple paths, but is only rewarded at the target if a particular route, the one associated with the tactile landmark, was chosen.

Single neuron recordings from different areas within the hippocampal formation during the performance of this task have been collected in six animals. In the initial analysis, we examined how task salience influences activity. Given that three points on the route are particularly salient to the task (the landmark site, the intersection, and the goal location), it was hypothesized that these areas may bias the firing patterns of neurons. Indeed, firing patterns of neurons in the dentate gyrus region were found to be biased by task salience, in that the number of cells with firing fields at the three salient locations is increased relative to the number of cells with firing fields at other locations. Further analyses will attempt to determine whether activity patterns in the dentate gyrus reflect a unique function for this hippocampal sub-region.

To date, the analysis of firing rate maps, i.e., the average firing rate of a neuron in relation to a circumscribed area of space, is both the bane and crux of most of the research done in this area. The undeniable relationship between a neuron's firing rate and space deserves a more systematic analysis than a simple firing rate map. Recently, we have employed information theoretic analyses to learn exactly how much information about space is contained within a neuron's response. In contrast to the usual firing rate maps, this analysis method considers response variance and exact conditional probabilities, and it does not one-sidedly consider increases in firing rate as necessarily more informative than decreases. Most reports detailing the spatial firing patterns of hippocampal "place cells" have labeled as aberrant those neurons with "multiple place fields." Now, by exploring the combination of this analysis and a multifaceted navigational task, we can shed some light on the details governing the complex firing patterns. One preliminary finding is related to the example provided above, as it often seems that "less is more" for many cells of the subiculum. This implies that, for many cells in this nucleus, spatially related decreases in firing are often more informative about space than increases in firing rate. (Douglas Nitz, William Kargo, Bryan Wilent; see publications 517, 534, 535, 536, 552)

Learning Processes That Enhance Cognition

Humans and lower animals maximize their ability to act appropriately in an environment by developing certain skills to a high level. A conductor for a symphony, for instance, uses his or her extraordinary skills in pitch and tempo discrimination to direct the actions of individual musicians. Attentional processes are certainly brought to bear in enhancing awareness of particular environmental features. However, our recent research indicates that persistent attention to particular items in the environment, in this case particular sounds, produces long-lasting changes in the activity profiles of neurons in auditory regions of the cerebral cortex. This work highlights the mechanisms by which attention mediates learning and which, in turn, alters awareness of the surrounding environment.

During early development, the brain establishes precise patterns of functional connections based largely on individual experience. In adulthood, the brain nevertheless retains a large capacity for adaptive modifications in response to interaction with the environment. This "experience-dependent plasticity" is considered to be a major basis for learning and memory, and it is crucial for recovery from brain damage.

Animals utilize the spectral information of sound to communicate, localize prey, and detect predators. The spectral information of sound is extracted, transferred, and represented in the auditory pathway from the ear to the cerebral cortex. In many areas of this pathway, neural responses are organized according to pitch, where nearby cells respond to similar frequencies (a "tonotopic" organization, or "map"). At present, it is unknown whether normal experience is required for maintaining this auditory map in adults.

It is well known that exposure of adult animals to high levels of noise or tones that can cause damage to the hair cells in the cochlea will induce reorganization along the auditory pathway, but it had not

been known whether or not such reorganization could take place even without extreme noise levels or cochlear damage.

To address these questions, we manipulated the acoustic environment of adult rats and examined their auditory maps using both immediate early gene *(c-fos)* immunocytochemistry (which reflects increased neural activity) and *in vivo* electrophysiology to monitor neural activity directly. Adult rats were exposed to low-intensity white noise (which does not damage the ears) continuously for 30 days. A profound effect of this noise exposure was revealed all along the auditory pathway.

Immunocytochemistry of *c-fos* protein demonstrated that the spatial representation of spectral information was significantly expanded, even at the level of the dorsal cochlear nucleus, the first structure where a tonotopic map is found along the ascending auditory pathway. The representation of the spectral information had also deteriorated throughout the central auditory pathway. Detailed mapping of neuronal electrophysiological responses also revealed a dramatic reorganization of the map in the auditory cortex: the tonotopic map was completely reorganized into frequency clusters. Thus, normal acoustic experience seems to be required for maintaining the map in the auditory cortex of adult animals, and even low-level sounds can result in plastic changes. These results also provide insights for understanding the neural correlates of noise-induced hearing losses that affect a large number of people. (Weimin Zheng; see publication 538)

Synaptic Electrophysiology

Neurons of the basal forebrain sporadically release the neuromodulator acetylcholine onto neurons of the cerebral cortex, the structure where our perception of the world and our responses to it are realized. Basal forebrain neurons degenerate in Alzheimer's patients who suffer from impairments in arousal, attention, learning, and memory. Similar symptoms are found in animals where lesions specific to acetylcholine neurons have been made. Recent work at the Institute connects the effects of acetylcholine on neurons of the cerebral cortex with learning processes of the cerebral cortex. This research brings together, into a coherent picture, the mechanism by which attention can enhance learning to produce efficient functioning in the environment.

Over the last two decades there has been a significant evolution in our understanding of the function of the primary motor cortex. Several lines of evidence indicate that it is not only responsible for directly controlling voluntary movements, but is also an important site for the plastic changes that underlie motor learning. In rodents, these include changes in number of connections, connection strengths, and firing patterns. How these changes are mediated at the cellular level is not understood, but it is clear that the neuromodulator acetylcholine is an important player. Chemical lesions of cholinergic input to the neocortex prevent skilled motor learning in rodents, and synaptic plasticity in the primary motor cortex is difficult to elicit in the absence of cholinergic (and specifically muscarinic) stimulation.

In vitro preparations (brain slices and cell cultures) allow the inner workings of neurons, and the changes they undergo, to be examined. Neurophysiological investigation using *in vitro* preparations has a long and impressive history, but scientists at the Institute have approached the topic in a fresh and useful way. The guiding idea is that, in order really to understand how neuromodulators such as acetylcholine affect neuronal response properties, one must try to recreate as closely as possible the conditions that neurons actually experience within intact brains. A central feature of those conditions is bombardment by a complex barrage of excitatory and inhibitory synaptic inputs, which provides the context in which cortical neurons respond. Background activity—by depolarizing neurons, increasing membrane conductance, and introducing fluctuations—strongly alters many aspects of neuronal responsiveness. We examined how this activity affected the response of motor cortex neurons to acetylcholine. The strategy was to marry computer simulations and biological neurons to simulate *in vivo*-like background activity in a brain slice. In this way, we were able to merge *in vitro* and *in vivo* approaches, retaining the many advantages of the slice preparation—especially precise knowledge of and control over inputs—while introducing important features of the *in vivo* condition.

The "dynamic clamp" feedback system was used to introduce *in vivo*-like inputs into pyramidal neurons in slices of forelimb motor cortex. The dynamic clamp method permitted the use of computer simulations to insert artificial synaptic or membrane conductances through a patch pipette into biological neurons, thereby creating a hybrid circuit of model

and real neurons making it possible to explore neuronal response properties under more realistic conditions. Investigating muscarinic stimulation in this way, we found that background inputs significantly reduce most previously reported muscarinic effects, including depolarization, plateau firing, and excitability, to the extent that their role should be re-evaluated. However, one muscarinic effect was well preserved: sensitivity to sustained, correlated inputs. These are important for signaling forelimb movements and for inducing synaptic plasticity changes that underlie learning of motor skills. (Niraj Desai, Elisabeth Walcott; see publications 537, 583)

Fragile X Syndrome

Fragile X syndrome is the most common inherited form of mental retardation and is an important cause of autistic-like behaviors. Efforts to understand its neural basis have focused on mouse models in which the transcription of a gene called *fragile X mental retardation (FMR1)* and its subsequent translation into the fragile X mental retardation protein (FMRP) have been disrupted. FMRP-knockout mice exhibit some of the same phenotypes as humans with fragile X syndrome, and so they are believed to provide a reasonable animal model in which to study mental retardation and its possible therapies. FMRP is thought to be important for synaptic transmission and neural plasticity—mice with alterations in the *FMR1* gene have changes in dendritic spine morphology and in certain kinds of synaptic plasticity—but its precise function remains unclear.

Our work investigates the basic electrophysiology and synaptic plasticity of pyramidal neurons in the sensorimotor cortex, using whole cell patch recordings in brain slices obtained from mice with altered FMRP expression.

In our initial experiments, we found that the absence of FMRP does not alter the basic electrical properties of these neurons. In particular, resting potential, conductance, spike threshold, and firing frequencies are unchanged. This perhaps makes sense because the knockout mice are viable; they do not die, nor are they completely unable to function. Rather, they have difficulty learning tasks that normal mice pick up with reasonable ease (like navigating a maze). This would suggest that it is neuronal plasticity which is modified, and thus our work has focused on two distinct forms of plasticity: long-term potentiation of intrinsic excitability (LTP-IE) and spike-timing-dependent plasticity (STDP). LTP-IE is intrinsic in expression and requires activation of one of the metabotropic glutamate receptors, mGluR5. STDP is synaptic in expression and requires activation of the N-methyl d-aspartate (NMDA) receptor for the neurotransmitter glutamate.

In the LTP-IE protocol, stimulation of mGluR causes the long-lasting suppression of a calcium-dependent potassium current. This current normally tends to reduce the ability of neurons to fire in bursts or in long, repetitive sequences. Its suppression then increases the excitability of individual neurons, which in turn can produce dramatic changes in network activity. Our results show that this form of intrinsic plasticity is not affected: the same amount of mGluR stimulation suppressed the calcium-dependent potassium current as strongly in the knockout mice as in normal mice. Our attention has now turned to studying how plasticity at the level of individual synapses may be affected in this animal model. (Niraj Desai, Peter Vanderklish; see publication 582)

Mechanisms of Gene Regulation in the Brain

A key area of neurobiological research is to study mechanisms of gene regulation in the brain, with particular emphasis on identifying DNA regulatory elements and transcription factors that control the expression of genes which regulate neurotransmitter systems. Changes in neuronal physiology resulting from behavior or the administration of pharmacological agents can lead to activity-dependent changes in gene expression in neurons. These changes in gene expression provide a basis for the adaptive alterations in neuronal physiology that accompany learning. Dopamine is a key neuromodulator of reward-based learning in the brain; thus it is important to identify the genes that are dynamically regulated by reward-based learning in neuronal circuits influenced by dopamine. These circuits include neurons of the striatum, the prefrontal cortex, and dopamine-producing neurons of the substantia nigra and ventral tegmentum. Several projects at The Neurosciences Institute involve identifying mechanisms by which particular psychoactive drugs regulate the genes that are involved in dopaminergic neurotransmission, particularly in the basal ganglia. The basal

ganglia have been implicated in a number of neurodegenerative diseases that affect movement, motivation, and motor control such as Huntington's chorea and Parkinson's disease. In addition to the dopamine system, we are interested in the mechanism that regulates a particular gene called MeCP2, which is the cause of a developmental neurological disorder in females called Rett syndrome. Our findings are summarized below.

Pharmacological regulation of dopamine receptors in the basal ganglia

Our studies have focused on identifying genes that are dynamically regulated by five drugs that affect signaling at dopamine receptors and adenosine receptors, both of which are important regulators of neuronal activity in the basal ganglia. The drugs are caffeine (an antagonist of the adenosine 2A receptor, A2AR), CGS 21680 (an agonist of A2AR), haloperidol (an antagonist of the dopamine 2 receptor, D2R), bromocriptine mesylate (BCM, an agonist of D2R and an anti-Parkinsonian drug), and clozapine (an atypical antipsychotic).

In previous work, researchers at the Institute showed that caffeine—the most widely used psychoactive drug in the world—stimulates transcription of the gene encoding the D2R in rodents and in cultured cells. More recently, we found that acute administration of clozapine and BCM also controls expression of dopamine receptors in neurons of the striatal portion of the basal ganglia. In contrast to caffeine, which increases D2R expression, clozapine and BCM were found to decrease D2R expression. Moreover, the combination of clozapine and BCM showed a synergistic and dramatic decrease in D2R expression, particularly in females (the increase in D2R expression that we previously observed with caffeine was also sexually dimorphic). Collectively, the results suggest that psychoactive drugs affecting the dopamine system have gender-specific effects on gene expression in the striatum.

Identifying mechanisms that protect dopamine neurons from cell death

A remarkable convergence of epidemiologic and laboratory data has supported the notion that the psychoactive drug caffeine reduces the risk of developing Parkinson's disease (PD) by preventing the degeneration of nigro-striatal dopaminergic neurons. Caffeine, along with other antagonists of A2AR, is known to protect dopaminergic neurons in several toxin models of PD. However, the cellular and molecular mechanisms by which the signaling by the A2AR contributes to neuronal death are not yet established.

Mitochondrial dysfunction is known to be an important cause of dopaminergic cell death in PD. To extend the analyses of genes that show altered expression in response to dopamine receptor signaling, a proteomic approach has been adopted. This involves using the technique of mass spectroscopy to identify proteins that rapidly change expression in the striatum of wild type mice in comparison to those in mice lacking the dopamine receptors D1R and D2R. This methodology has been used to identify proteins that show alterations in expression in response to caffeine, CGS21680, BCM, and the antipsychotic drug haloperidol. In preliminary work, several proteins that are regulated by dopamine have been identified. One is a subunit of cytochrome c oxidase, the rate-limiting enzyme of the mitochondrial electron transport chain involved in adenosine triphosphate (ATP) synthesis. These findings have focused our attention on understanding how metabolic state and electrophysiology in the neuron influence each other. Current studies are directed at altering the expression of cytochrome c oxidase in neurons in order to determine how the metabolic state alters cell physiology and survival in the face of neurotoxicity.

The death of neurons and progression of PD are accelerated by oxidative stress that generates reactive oxygen species (ROS), which induce apoptotic cell death in dopamine neurons. Since apoptosis of dopaminergic neurons is suppressed by antioxidants, generation of ROS in neurons may initiate the process of cell death, and antioxidant therapy may delay the decline of dopamine neurons in the brain. Based on this hypothesis, we are studying free radical scavenging and antioxidant activities of caffeine. We are also analysing its effects on activating native antioxidant and neuroprotective pathways that involve the native antioxidant glutathione (GSH) and the enzymes catalase and superoxide dismutase (SOD). The levels of GSH, catalase, and SOD are subject to regulation by drugs that both stimulate and antagonize A2AR and D2R; this regulation may be the principal mechanism of neuroprotection by caffeine.

Metabolic stress is an important trigger factor for the neurodegenerative process of PD. In particular, reduced activity of mitochondrial respiratory chain complex I (CXI, also known as NADH: ubiquinone reductase) has been consistently found in PD patients. However, the pathophysiological events that are induced by CXI inhibition and that lead to the selective degeneration of dopaminergic neurons are unknown. Nevertheless, recent studies indicate that the concentration of ATP in the cytoplasm of neurons has a crucial role in the control of metabolic activity in these cells by regulating the activity of ATP-sensitive potassium channels (KATP). Measurements of the cytoplasmic ATP concentration are therefore of major importance for evaluating the connection between energy metabolism, neuronal physiology, and cell death in dopaminergic neurons.

KATP channels are of special interest because the probability of their being open directly depends on the metabolic state of a cell. KATP channels are closed at high ATP/ADP ratios and open in response to decreased ATP and increased ADP levels. By means of this mechanism, KATP channel activity exerts a powerful control on neuronal excitability. It is tempting to speculate that, in PD, a chronic reduction of neuronal activity might not be neuroprotective by reducing ATP consumption. Rather, it might lead to a reduced expression of activity-dependent genes that promote survival, such as those for neurotrophins. In this scenario, transient KATP channel activation is a short-term neuroprotective response to metabolic stress, but chronic KATP activation could have fatal consequences for dopaminergic neurons.

Based on these considerations, we are using rat PC12 cells and human neuroblastoma SH-SY5Y cells to study the relationship between cell metabolism and physiology. These experiments involve analysis of the cellular concentration of ATP in response to drugs that affect A2AR, D2R, and mitochondrial ATP synthesis. The drugs include caffeine, CGS21680, BCM, haloperidol, and rotenone (an inhibitor of CXI and opener of KATP channels). Our studies also involve assessing free radical scavenging and antioxidant activities in response to these drugs, as well as their effects on glutathione (GSH), catalase, and superoxide dismutase (SOD) activity. Mass spectroscopy will be used to identify changes in protein expression in neurons that are correlated with alterations in metabolism that are induced with these drugs. The overall goal is to define the molecular components that function in the alteration of cellular metabolism under conditions of cell death and neuroprotection.

The molecular biology of Rett syndrome

Rett syndrome (RTT) is a neurological disorder involving cognitive and motor dysfunction in young females. It results from mutations in the gene encoding the methyl-CpG-binding protein 2 (MeCP2). MeCP2 is a transcriptional repressor, and it has been proposed to silence the transcription of genes by binding to methylated cytosines within their promoters, subsequently recruiting co-repressor protein complexes containing Sin3A and histone deacetylases.

Despite the widespread expression of MeCP2 in both neuronal and non-neuronal tissues, symptoms of RTT are largely neurological. In the central nervous system, MeCP2 is expressed exclusively in neurons. Moreover, targeted deletion of the MeCP2 gene in the central nervous system of mice is sufficient to produce the symptoms of RTT, suggesting that neuronal expression of Mecp2 is crucial for normal development and function of the brain. Mild overexpression of MeCP2 (2-fold higher than the levels normally observed in wild-type mice) caused profound motor dysfunction. These findings reveal the necessity of precisely regulating the level of MeCP2 protein expression *in vivo* in order to sustain normal neurological function. Although numerous mutations in the MeCP2 gene have been identified in RTT patients, there is little understanding of how these mutations lead to the clinical manifestation of the disease. Moreover, there is currently no treatment for RTT. Understanding the mechanism that regulates the expression of MeCP2 in neurons throughout development is an important step toward designing viral vectors that might be used in gene therapy approaches to treat RTT patients.

Our recent work has identified the regulatory region of the mouse MeCP2 gene that is sufficient for its restricted expression in neurons. A segment of the MeCP2 gene (containing nucleotides from positions -677 to+56) exhibited strong promoter activity in neuronal cell lines and cortical neurons but was inactive in non-neuronal cells and glia. The region necessary for neuronal-specific promoter activity was then localized to a smaller portion (nucleotides -63 to -45). Several nuclear factors were found to bind to this region, and some of these factors were enriched in nuclear extracts prepared

from the brain. To examine the activity of the MeCP2 promoter *in vivo*, transgenic mice expressing the LacZ reporter gene driven by the –677/+56 region of the MeCP2 gene were generated. The transgene was expressed in the mesencephalon as early as embryonic day ten (E10), and in the hindbrain and spinal cord by E12. Interestingly, a marked induction of transgene expression was observed postnatally throughout the brain, similar to that of endogenous MeCP2. However, expression of the transgene was absent in non-neuronal tissues that are known to express MeCP2. Taken together, these data indicate that the –677/+56 region of the MeCP2 promoter partially recapitulates the native expression pattern of the MeCP2 gene. These studies provide an important first step in designing vectors for gene therapy applications in which exogenous MeCP2 might be introduced in RTT patients. (Fred Jones, Megumi Adachi, Jie Jing; see publications 516, 531, 549, 572, 581, 590)

Neurodegenerative Disease

An emerging theme in the study of progressive neurodegenerative diseases is the connection between oxidative stress, mitochondrial function, and protein degradation. How these aspects of cell function are connected, and the sequence of events leading to neuronal death, are not understood. Long term neuronal cell cultures derived from specific areas of the central nervous system that are affected in neurodegenerative diseases have been established to study this question. Recombinant lentiviruses have been used as a way for efficient delivery of genes that may be critical to the underlying disease mechanism. Two areas of research have been pursued.

The first project involves an ongoing study of the response of midbrain dopamine neurons to oxidative stress. Oxidative stress has been implicated in the demise of dopamine neuron in Parkinson's disease. It is now clear that, under the culture conditions used, removing exogenous antioxidants leads to signs of oxidative stress in dopaminergic neurons. This is evidenced by an increase in the amount of the lipid peroxidation product, hydroxynonenal. At the same time, there is an increase in the expression of the inducible antioxidant enzyme, heme oxygenase 1, but a selective decrease in the amount of manganese superoxide dismutase (SOD2) in dopaminergic neurons. SOD2 is the enzyme that is solely responsible for removing superoxide anions from the matrix of mitochondria. There was no significant loss of dopamine cells in cultures deprived of exogenous antioxidants for longer periods of time, suggesting that the decrease in SOD2 may constitute an adaptive response by dopaminergic neurons to oxidative stress.

Using a mitochondrially directed dye that is specifically oxidized by superoxide, cells expressing lower amounts of SOD2 were also shown to contain less superoxide. This is consistent with the idea that the decrease in superoxide production may be the result of a partial uncoupling of the mitochondria in dopaminergic neurons. High levels of glucose are present in the growth medium, which are likely to reduce the dependence upon ATP generated by oxidative phosphorylation. In future experiments, the level of exogenous glucose will be varied to try to determine whether dopamine neurons are functionally impaired under conditions of chronic oxidative stress as a result of reduced energy production by mitochondria. Over-expression of SOD2 might be thought to be beneficial under conditions of oxidative stress, but our results indicate that long-term over-expression of SOD2 is in fact deleterious to neurons under these conditions. This may be due to the generation of more hydrogen peroxide, the product of superoxide dismutase activity, and the resultant formation of highly toxic hydroxyl radicals via the Fenton reaction. Co-expression of SOD2 and either peroxidase or catalase, engineered to enter the mitochondrion, will be tested to analyze this further.

A second project focuses on the signal transduction pathways that govern the response of cells to oxidative stress. The protein kinase AKT is a key player in such regulatory pathways. Our work showed that expressing a constitutively active form of this enzyme in midbrain neurons drives down SOD2 and drives up expression of heme oxygenase in the absence of oxidative stress, indicating that AKT may control the response of dopaminergic neurons to oxidative stress. AKT is one of several stress-activated protein kinases that regulate the activity of FOXO proteins, which are members of the forkhead class of transcription factors. Work by many groups has shown that phosphorylation of FOXO proteins (FOXO1, FOXO3) by AKT decreases DNA binding, causes their exclusion from the nucleus, and promotes proteasome-mediated degradation. In the context of cell survival, this negative regulation

of FOXO transcriptional activity by AKT appears to be a double-edged sword. On the one hand, it has been shown that FOXO directly regulates the transcription of key components of both the extrinsic and intrinsic apoptotic pathways. On the other hand, it has been shown that FOXO is a positive transcriptional regulator of SOD2 and catalase.

To explore the role of FOXO proteins in neuronal survival, our work has used a novel virus that encodes a fusion protein between green fluorescent protein and FOXO3A. Using this fluorescent fusion protein, FOXO expression in living cultures can be analyzed. In a set of live imaging experiments using hippocampal neurons and selective protein kinase inhibitors, the distribution of FOXO between nucleus and cytoplasm was regulated by AKT activity in some neurons, but by another protein kinase, IKK, in other neurons. Not surprisingly, this suggests that different signal transduction pathways are active in different populations of neurons. (Geoffrey Owens, Edward Keefer; see publication 513)

Developmental Neurobiology

Homeobox proteins in muscle development

Contemporary studies use a combination of developmental genetic techniques and molecular analyses to identify regulatory mechanisms and factors that promote plasticity of terminally differentiated mammalian cells. Much remains to be learned about the factors that control the morphological plasticity of differentiated cells.

We found that the homeobox gene *Barx2* has a very interesting pattern of expression. Early in development, *Barx2* is expressed in developing cartilage, muscle, and brain. Later its expression is almost completely eliminated from terminally differentiated tissues but persists in the tissues that undergo proliferation and differentiation during adulthood. Thus *Barx2* expression is found in developing joints and articular cartilage, in the subventricular zone of the brain, and in regenerating muscles. We found that *Barx2* accelerates differentiation when expressed in undifferentiated myoblasts. In contrast, in differentiated myotubes, *Barx2* expression induces dedifferentiation. Thus, *Barx2* may participate in muscle regeneration by inducing dedifferentiation of myotubes into proliferative myoblasts and redifferentiation of this pool to generate new muscle fibers. In support of this notion, it was found that *Barx2* is expressed in the regeneration zone after embryonic digit tip amputation, and it promotes cell proliferation. Together, these data suggest the hypothesis that *Barx2* influences both embryonic muscle differentiation as well as muscle regeneration. Underpinning this hypothesis is the idea that homeobox transcription factors can either positively or negatively regulate muscle gene expression in different cellular contexts. These studies help define the role of *Barx2* and other homeobox proteins in tissue plasticity and regeneration and allow us to explore the molecular mechanisms of its differential activity.

Epidermal and fibroblast growth factors in the development of neuronal diversity

The mechanisms underlying the development of neuronal diversity are largely unknown. In the mammalian cerebral cortex, gamma-aminobutyric acid (GABA) neurons represent 10-25% of all neurons. Neuronal cultures prepared from rat embryonic cerebral cortex at embryonic days 16-18 form two different populations of GABAergic neurons. A distinct population of large GABAergic neurons was found in the cultures just after the plating. This population differentiates into large neurons with extensively developed and branched dendritic trees and is necessary for generation of synchronous oscillatory network activity. The ability of these neurons to differentiate even in the presence of the mitogen inhibitor ARAC suggests that most of these neurons are post-mitotic. Neurons in the second population of GABAergic cells are much smaller, are predominantly bipolar in appearance, and form clusters between the processes of large neurons. Small GABAergic neurons are generated during the first week in culture; their function and the mechanisms underlying their differentiation are unknown. Further characterization of small GABAergic neurons showed that they extensively express epidermal growth factor receptor (EGFR), while the expression of fibroblast growth factor receptor (FGFR) is low. In accord with this finding, the application of FGFR inhibitor SU5402 to the cortical cultures just after plating had little effect on differentiation of small GABAergic neurons while treatment with EGFR inhibitors PD153035 and ZD 1839 almost completely eliminated population of small GABAergic neurons. These treatments did not affect the differentiation of large GABAergic neurons, but application of the

EGFR inhibitors disturbed synchronous oscillatory network activity of these cells. These findings suggest that epidermal growth factor may be involved in differentiation of cortical neuronal subpopulations later in development.

Protocadherins in regulation of mouse nervous system development

Studies have been carried out to define the expression pattern of Pcdh8, a member of a new delta family of protocadherins. Delta-protocadherins constitute a group of cadherins characterized by several conserved motifs in their cytoplasmic domains. Some of these protocadherins are expressed in several isoforms, the functions and expression patterns of which are not known. An analysis of the expression of short and long variants of Pcdh8 during early mouse development, using RT/PCR and *in situ* hybridization, showed that both isoforms were predominantly expressed in the nervous system, and that their expression patterns appeared to be developmentally regulated. However, there was a difference in the expression pattern of these isoforms. The long isoform of Pcdh8 is expressed in the central nervous system, while the short isoform is found in both neuronal and non-neuronal tissues. The differential expression of these alternative cytoplasmic domain variants suggests that Pcdh8 may regulate cell adhesion in a variety of developmental processes and that this may involve different intracellular interactions. Studies of the regulation of Pcdh8 expression showed that the conserved neuron restrictive silencer element (NRSE) binding domain for repressor element-1 transcription factor/neuron restrictive silencer factor (REST/NRSF) was present within the first exon of Pcdh8 and other members of delta family of protocadherins. It has been reported previously that REST/NRSF is a critical regulator of the neuronal differentiation pathway. Consistent with this finding, our experiments showed that the expression pattern of Pcdh8 is affected in mice lacking this transcription factor.

Reactive oxygen species in neuronal differentiation

Populations of cells from embryonic rat cortical cultures were sorted, using a fluorescence-activated cell sorter, based on the levels of a group of chemically related oxygen derivatives called reactive oxygen species (ROS). High ROS-producing cells expressed the neuronal marker beta III tubulin, did not incorporate BrdU or express nestin, while low ROS producing cells incorporated BrdU, expressed nestin, and when cultured at a clonal density formed neurospheres that could be further differentiated into neurons, oligodendrocytes, and astrocytes. To answer the question whether ROS levels are important for neuronal differentiation, ROS levels were decreased by the exogenous addition of superoxide dismutase and catalase. These enzymes did not alter the overall number of neurons differentiated from clonal neurospheres, but they changed the ratio of two neuronal types found in neurosphere cultures. Thus in superoxide dismutase- and catalase-treated cultures, the number of neurons with small cell bodies, shorter processes, and nuclear expression of the protein calretinin was increased, while the number of neurons with large cell bodies, highly branched multiple processes, and cytoplasmic expression of calretenin was decreased. (Helen Makarenkova, Kathryn Crossin, Robyn Meech, David Edelman, Elisabeth Walcott, Geoffrey Owens; see publications 548, 568, 579, 586, 587, 588)

Gene Networks and Behavior in *Drosophila*

The intricate network of gene interactions in an organism provides a compelling example of biological complexity, comparable in many ways to that of the brain. Not only are the two kinds of network comparable in many ways, but they also have a very intimate relationship in the extent to which gene action is a necessary prerequisite to brain function and behavior. The problems of interaction in gene networks and the manner in which gene networks influence behavior are being addressed in studies of the fruit fly, *Drosophila melanogaster.*

A gene network surrounding the Syntaxin1A *locus*

Interactions among genes are key to understanding the realization of any phenotype. First championed by Sewall Wright as part of his evolutionary theory, the study of gene interactions and networks has been integral to quantitative genetics, to classical mutant analysis, to molecular genetic analysis, and more recently to the interpretation of DNA microarray results. In the current molecular paradigm, this viewpoint has assumed that the elements of a gene network are relatively specific in their interactions and that the relationships among them are stable.

The first assumption—specificity—has come under increasing scrutiny as more and more genes are found to be pleiotropic (wide-ranging in effect), a property that appears to be particularly prevalent in genes implicated in neural function and behavior. The second assumption—the stability of relationships in a gene network—is one we have also begun to question as the result of our analysis of interactions among a set of genes affecting loss of coordination in *Drosophila*. A classical analysis of epistasis was performed on sixteen mutations isolated as enhancers or suppressors of temperature-induced coordination loss by a mutation of *Syntaxin-1 (Syx-1A),* an essential component of the machinery of secretion and synaptic transmission. The functional relationships among the 16 genes were shown to vary with their genetic context, changing substantially depending on the presence or absence of the *Syx-1A* mutation.

Further genetic analyses on a subset of the *Syntaxin* interacting variants were performed to test the replicability of phenotypes originally reported. The result was the interesting finding that whereas absolute values showed some deviation from previous findings, the relationships among the network elements was highly correlated and reproducible. This is encouraging, as it indicates that fluctuations due to environmental variables do not disturb the network interactions we are interested in.

Further statistical analysis has also been performed on the original pair-wise interactions in order to assess the effect of network perturbation on the degree of deviation of observed from predicted phenotypes. The results of our analysis revealed that an impaired network (i.e., one with the major mutation in *Syx1A* gene) shows a decreased range of possible phenotypes. We were interested in determining whether degeneracy—the yielding of similar outputs from different structural interactions—operated in these networks. Our observations have great significance for the idea that degeneracy in networks confers greater robustness on them and gives us an opening to probe what aspects of degeneracy are particularly important.

A broad-based matrix study of gene networks and behavior

The inference from our previous studies is that gene networks ramify widely and that behavior is very sensitive to changes, even subtle ones, in these networks. To address this issue more directly, we have carried out an experiment designed to ask how sensitive various behaviors are to perturbations anywhere in the genome. Can every phenotype be affected, at least quantitatively, by any change in genotype? As a starting point, eight mutations were chosen to be unrelated in biochemical function and in phenotype, placed on a common genetic background, then tested in all pair-wise combinations of double heterozygotes and assayed for phototaxis, geotaxis, locomotor activity, sleep/wake cycle, mating speed, courtship song, circadian rhythms and temperature-induced paralysis.

Preliminary behavioral results indicate that it is primarily the epistatic interactions that show specificity with respect to behavioral phenotype, suggesting that multi-gene interactions are powerful modifiers of behavioral phenotypes.

A gene/metabolic network surrounding the foraging locus

To delve into the interplay between gene and metabolic networks, analysis has been performed to compare changes in the expression of mRNA and small molecule metabolites between the two naturally occurring alleles of the *foraging* locus: *Rover* and *sitter*. The large number of genes and metabolites differing between these two strains suggest several different possible etiologies for the *Rover/sitter* difference, one of which is that those carrying the *sitter* allele are in a mild state of mitochondrial dysfunction. Our work has shown that the two alleles are distinguished by a 12% difference in expression level of the cGMP-dependent protein kinase encoded by the *foraging* gene, and the current results indicate that a change as subtle as this one can have wide ramifications throughout biological networks.

Gene network changes after phenotypic selection

Genetic selection experiments are a traditional way of assessing natural variation affecting a trait. In recent years, we have pioneered the use of DNA microarrays as a means of analyzing the complex changes in gene activity resulting from multi-generational selection for behavioral traits. Two different behaviors, aggression and geotaxis, have been studied.

Aggression

A selection paradigm has been developed to increase aggressive behavior in *Drosophila*. In as

few as ten generations, lines selected for aggressive behavior became dramatically more aggressive than control lines as measured with a novel, two-male arena assay. Aggressiveness increased in subsequent generations of selection. Array analyses of gene expression at generation 21 of selection pointed to a number of candidate genes that showed differential expression in the aggressive and control lines. Mutations in some of these genes directly affected aggressive behavior.

Further work on these strains has focused on genes whose level of expression declined significantly during the course of selection for greater aggression. The rationale for focusing on genes with lower levels of expression is that this allowed us to test independently obtained mutants for similar effects. Since mutants generally have lower levels of expression of the mutant gene, if a gene is critical, the mutant would be expected to mimic the aggressive phenotype. Mutant strains exist and are being tested; two strains of particular interest carry mutations in a multi-vitamin transporter gene and in a cytochrome P450 gene.

This work has also addressed the question of whether serotonin has any role in aggression in the fly. Comparing tissue from the heads of flies from the aggressive and control lines, microarray analysis revealed no differences in expression in any of the genes that are related to serotonin synthesis and signaling. Similarly, direct measurement of serotonin levels in the heads of the selected populations did not reveal any significant differences, further suggesting that global changes in serotonin level do not play a role in the selected response. To evaluate whether or not serotonin can affect aggressive behavior (as has been shown in other invertebrates but not insects), serotonin levels in the selected flies were artificially manipulated by feeding them drugs that either act as a precursor of serotonin or inhibitor of serotonin metabolism. The serotonin precursor 5-hydroxytryptophan increased the levels of serotonin (5-hydroxytryptamine) in the brain by roughly ten-fold, and the inhibitor of serotonin synthesis, alpha-methyltryptophan, decreased the serotonin levels in the brain approximately two-fold. All the lines treated with 5-hydroxytryptophan showed significantly increased aggression frequencies over untreated flies, while flies treated with the serotonin synthesis inhibitor showed only a modestly decreased fighting frequency. Increases in serotonin affected the intensity of fighting as well as the frequency of escalations in intensity (the most intense fighting between flies). These results clearly suggest a role for serotonin in fly aggression, but they also suggest that a mechanism involving serotonin was not the basis for selection in these lines.

Geotaxis

Our previous work analyzed strains of *Drosophila* selected over many generations for divergence in their response to gravity ("hi" strains vs. "lo" strains). After sampling approximately 40% of the genome, differences in expression between the two strains were found in a wide variety of genes. They included some involved in neuronal cell fate determination, in neuronal signaling, in circadian rhythms, and in basic cell biological mechanisms. Pre-existing mutations in these genes were also tested behaviorally. Some of the mutants were able to mimic the behavior of the selected lines, thus revealing the functional relevance of the expression differences in those genes for the selected phenotype.

Subsequent to that work, new strains have been derived containing subsets of the "hi" and "lo" genomes. These include strains in which single chromosomes from each genome is placed onto a standard (Canton-S) background, as well as recombinant inbred lines made from a series of hybrids. Behavioral scores from these strains indicate a high level of epistatic gene interaction. For example, a chromosome from the "hi" line can induce a "lo" response on a different genetic background. We are currently analyzing whole-genome DNA microarray results from these inbred strains to trace the sets of genes underlying the epistasis. These results suggest that the genetic basis for complex behavioral phenotypes is accessible to the kind of analysis previously reserved for relatively severe, single-gene mutations. (Bruno van Swinderen, Herman Dierick, Rozi Andretic, Daniel Toma, Ralph Greenspan; see publications 509, 564)

Sleep and Social Interactions in *Drosophila*

Previous work at the Institute demonstrated that fruit flies sleep, as judged by a number of criteria that would be applied to determine sleep in mammals. More recent analyses have demonstrated that sleep

behavior shows considerable variability both within and between individuals and populations of flies. Individuals reared in or exposed to sensory deprivation conditions are significantly shorter sleepers than their isogenic but socially stimulated siblings. This change in sleep amount is stable and depends up prior interactions with conspecifics mediated via the visual and olfactory (but not auditory) sensory modalities. Moreover, sleep amount varies linearly with the total number of flies that make up the social group to which the individual was previously exposed, suggesting a causal relation between the intensity of social stimulation during prior waking and subsequent sleep. (Indrani Ganguly and Paul Shaw)

Dopamine and Arousal in *Drosophila*

We have shown that methamphetamine (METH) prevents sleep in flies by consolidating wakefulness and increasing locomotor activity. This effect is at least in part dependent on dopamine levels, since an inhibitor of dopamine synthesis promotes sleep. METH also affects courtship behavior by increasing sexual arousal but decreasing successful sexual performance. To further investigate dopaminergic effects on brain function, electrophysiological recordings were made from the medial protocerebrum of wild-type flies. The results demonstrated that METH ingestion has rapid and detrimental effects on a specific brain response associated with perception of visual stimuli (see below). Recordings from genetically manipulated animals show that dopaminergic transmission is required for these responses, and that deficits in visual processing caused by attenuated dopaminergic transmission can be rescued by METH.

These studies showed that changes in dopamine levels differentially affect arousal for behaviors of varying complexity: complex behaviors degrade when dopamine levels are either too high or too low, and simpler behaviors show graded responses that correlate with changes in dopamine levels. (Rozi Andretic, Bruno van Swinderen, Ralph Greenspan; see publications 540, 573, 580)

Arousal and Visual Perception in *Drosophila*

Arousal, or behavioral responsiveness, covers a spectrum of different behavioral states, from coma and general anesthesia to sleep, wakefulness, and selective attention. In selective attention, we direct our attention to restricted portions of the veritable flood of inputs to our senses, and we use this filtered information to act appropriately. Research at the Institute revealed that the brains of organisms as relatively simple as the fruit fly *Drosophila* also contain mechanisms to filter sensory information and thereby produce biases in decision-making. We have developed methods, using *Drosophila*, to study and dissect the mechanisms supporting various levels of arousal and, in particular, behaviors analogous to selective attention to visual stimuli.

Neural correlates of arousal states are determined by recording local field potentials (LFPs) from the brains of behaving fruit flies. The flies are tethered to a post by their head and thorax, but they are free to move their legs, wings, antennae, proboscis, and abdomen. As such, they are free to perform many behaviors which are key to monitoring arousal states, all while LFPs are recorded from their brains.

Sleep experiments have revealed that LFP activity in the one to one hundred Hz range decreases in power while animals are quiescent and have increased arousal thresholds to automated mechanical stimuli. Periods of decreased correlation between brain activity and the fly's own movement precedes such quiescence. Ongoing changes in coupling between brain activity and movement also appear to determine whether flies will respond to the mechanical stimuli even while awake, suggesting that a general arousal effect is being measured.

Visual perception is studied by suspending the same recording preparation in a wrap-around arena with light-emitting diodes so that programmed virtual objects can be presented to the fly, along with other stimuli such as heat or odors. In this paradigm, a specific LFP response in the 20-30 Hz frequency range was found to be associated with visual salience effects, upon presentation of novel visual stimuli, and when odors or noxious heat were associated with visual stimuli. The 20-30 Hz response to salience displays attention-like qualities, as it is suppressed for other visual stimuli to which flies are unresponsive. In addition, multichannel recordings from different areas of the fly brain reveal that LFP coherence increases during such visual selection, as occurs during selective attention in human preparations.

Behavioral measures of visual perception in fruit flies are determined by two different approaches, one precise and fine-grained, the other high-throughput and amenable to genetic screens. In the first instance, a virtual-reality system is used where feedback from a fly's flight behavior controls the position of virtual objects in the arena. In this way, animals are able to "report" their perception of objects as well as their discrimination among objects. This approach, which complements LFP salience experiments, can be used in combination with brain recordings. The second method of determining visual responses in flies involves running populations of flies through an "optomotor" maze, in which flies are presented with a moving pattern of dark and light stripes. Normally, flies will spontaneously run in the same direction as the flow of the stripes. Thus visual perception, optomotor responses, and attention-like effects can be efficiently quantified for *Drosophila* strains prior to selecting key lines for further characterization by electrophysiology. Responses in the optomotor maze were found to complement electrophysiological results obtained in identifying visual attention deficits in key mutant strains.

The vast genetic toolbox offered by *Drosophila* is used in order to study mechanisms supporting the arousal states identified in this organism. These tools include a large number of available mutant strains, as well as efficient mutagenesis techniques. Some of the most interesting strains with deficits in visual discrimination have been found in *Drosophila* variants that were isolated as learning and memory mutants. By controlling gene expression levels as well as neuronal function with specificity, the circuits responsible for arousal phenotypes in the fly are being defined. These experiments make use of the GAL4 expression system coupled to the temperature-sensitive *shibire* allele or a caged-ATP-purinoceptor construct to transiently attenuate or activate activity in specific neuronal groups in different regions of the fly nervous system. Together with classical mutant analysis, these approaches allow for a systematic dissection of the mechanisms required for assigning visual salience to objects as well as for maintaining other arousal states. (Bruno van Swinderen; see publications 545, 555)

Research Publications

The following publications resulting from research at The Neurosciences Institute have appeared since the previous edition of the *Scientific Report*. The list includes detailed reports on specific research as well as conceptual and review articles. Publications are listed in chronological order and numbered sequentially for ease of reference.

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