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During the past fiscal year faculty recruitment for the Institute has been completed: 3 professors, 1 associate professor, 12 assistant professors, and 5 research associates. In addition, 16 post-doctoral fellows and 13 technicians have been recruited, and the administrative staff has been hired. Laboratories for most of the faculty are now fully equipped and functional; the others are rapidly nearing completion. A 7 Tesla experimental NMR facility is now operational and a human 1.5 Tesla magnet has been ordered to support the research of the human cognitive group. Researchers are divided into 5 divisions: cognitive neuroscience (human); cognitive neuroscience (animal); computational neuroscience; drug discovery and design; and molecular neurobiology and plasticity. In addition, the Institute supports 6 complementary projects involving other faculty at Georgetown University Medical Center. During this period, a total of 53 papers and 43 abstracts were published by Institute faculty

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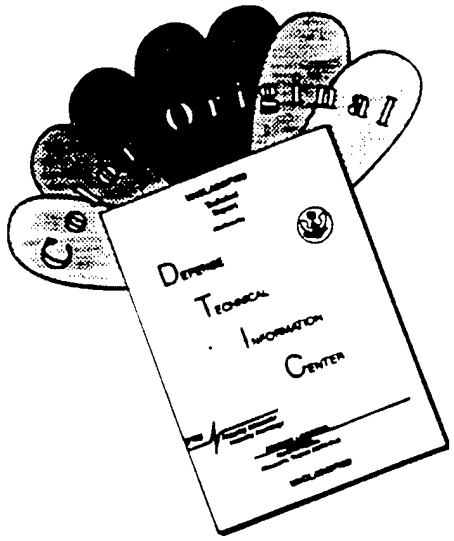

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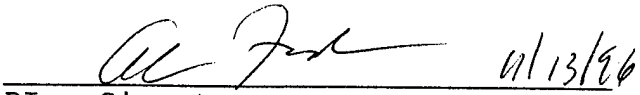

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For Georgetown University

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INTRODUCTION

The Georgetown Institute for Cognitive and Computational Sciences (GICCS) is a neuroscience research institute whose mission is to understand higher cognitive function through interactive collaborative efforts among scientists using multidisciplinary investigative strategies. Its major focus areas are: higher auditory processing and language; and brain injury and plasticity.

Of the three main sensory systems, hearing is the least well understood. GICCS has begun a major initiative to elucidate the complex mechanisms of higher auditory processing. Species with specialized hearing, such as bats, are used as models for complex sound processing and compared to those using cats and primates, which also use acoustic signals as a primary means of communication. Parallel research in humans using functional brain imaging and cognitive psychology will examine how the human brain deals with complex sounds, particularly those relating to speech. These studies address not only normal language processing but also examine disorders of speech/language, including developmental and acquired dyslexias. One goal will be to develop treatments for these disorders through specialized training.

Understanding and modifying brain plasticity represents another major research area. Investigators use tools from cellular/molecular neurobiology and from systems neuroscience, to study plasticity after acute or chronic brain injury as well as after early vision or hearing loss. This includes development of pharmacological strategies to limit brain damage and to enhance cognitive function after injury or neurodegeneration. Brain magnetic resonance imaging and spectroscopy are also used, employing a high field animal research magnet as well as human MRI, to clarify mechanisms of tissue damage and plasticity, and the response to targeted treatments. In addition to advanced brain imaging, computational neuroscience is an important experimental area that serves to integrate these multidisciplinary research efforts. Sophisticated computational methods are used to model sensory processing based upon the experimental studies. Predictions based upon mathematical modeling are evaluated in subsequent laboratory experiments.

Together, the goal of this diverse but complementary research team is to better understand cognitive processes in order to address important clinical problems including deafness, language disorders, traumatic and ischemic brain injury, and Alzheimer's Disease.

During the past fiscal year we have completed recruitment of faculty for GICCS as well as the development and instrumentation of their laboratories. Faculty include three at the professorial level, one associate professor, 12 assistant professors, and 5 research associates. A total of 16 postdoctoral fellows and 13 research technicians have also been recruited to date. The faculty are divided into 5 sections: cognitive neuroscience (human); cognitive neuroscience (animal); computational neuroscience; drug discovery and design; and molecular neurobiology and plasticity. There are numerous collaborative connections within and across these sections. For example, all members of the human cognitive group and several members of the animal cognitive group share a common interest in the use of magnetic resonance imaging technology to address fundamental questions. Magnetic resonance technology also links the animal cognitive group with the group in molecular biology and plasticity. The drug development and design group has extensive collaborations both within the Institute as well as involving other departments at the medical center. Six additional projects are funded through the Institute with other neuroscience faculty in the areas of complementary interest. Research developments during the past year are divided into 6 parts, one relating to each institute section and one relating to collaborative projects within Georgetown University Medical Center.

HUMAN COGNITIVE NEUROSCIENCE: There are four faculty members who are grouped into this area. Dr. Bavelier examines neural bases of visual cognition. Dr. Eden uses functional neuroimaging to study the pathophysiology of developmental dyslexia. Dr. Friedman examines the neuropsychology of language. Dr. Ullman investigates neural bases of language and memory. In addition, Drs. Pekar and Rauschecker conduct research projects in Human Cognitive Neuroscience using fMRI.

DAPHNE BAVELIER, PH.D.

Brain and Vision Laboratory

Introduction

The primary goal of my laboratory is to characterize the cognitive and neural processes that enable the visual system to segment a visual scene in discrete objects and maintain representations for these objects over time.

An impressive body of work from psychophysics and neuroscience has shown that the visual system initially decomposes a scene into separate features such as color, luminance, motion, edge orientation etc. Thus, when driving on the highway behind a green mini-van and a red sport car, separate sub-systems within the visual system process the colors and the shapes of these cars. These two features must, however, be properly recombined to preserve the right association of color and identity. Otherwise, we would be just as likely to perceive a red mini-van and a green sport car, rather than the other way around. Our work focuses on the mechanisms responsible for this integration of early visual features. Furthermore, we also assess how the representation of an object is maintained over time even in the presence of great variations at the sensory level. Hence, even if the red sport car disappears behind another car, we have no problem perceiving it as the same car when it reappears. The ease with which we select and track objects in a cluttered, ever-changing visual environment is in sharp contrast with the difficulty we face in designing artificial systems with such a skill.

This issue is addressed using a variety of experimental techniques. We use behavioral testing to identify the processes at play when viewing and orienting in a complex visual environment. In addition, rely on functional imaging (Event Related Potentials, ERP, and functional Magnetic Resonance Imaging, fMRI) to constrain our hypotheses and assess the neural systems underlying these processes. These techniques are used on control subjects as well as individuals with altered visual functions. More particularly, we are testing the hypothesis that the high level visual functions we study are enhanced in congenitally deaf individuals, and that this behavioral enhancement is linked to the functional re-organizations of a number of cortical areas.

Methods and Results

Characterization of the Cognitive Processes Underlying Visual Selection and Visual Tracking of Objects

Previous research has identified a number of principles, also termed Gestalt principles, that initially guide the recombination of early visual features corresponding to the same object. To lead to a durable precept of object, however, this initial recombination needs to be stabilized into visual memory as a representation specific to that object. While the 'Gestalt' process is automatic and applies in parallel across the visual field, the latter is a demanding process constrained by limited capacity resources. Thus, only a few object-specific representations can be set up in memory at a time (present data suggest a limit of four or so). Furthermore, if two different objects share the same feature, they will compete for integration of that feature in their respective memory representations. As a result it should be difficult to establish memory representations for objects that share similar visual features. Accordingly, we have shown that subjects have difficulty seeing two chairs when briefly presented with a visual display containing two visually identical chairs, a phenomenon termed repetition blindness (Kanwisher, 1987; Bavelier and Potter, 1992).

Our working hypothesis is that object-specific memory representations are essential for guiding behavior. As such they should mainly encode the information necessary for the viewer at that moment. Subjects did not show repetition blindness when presented with two pictures of different looking monkeys and told ahead of time to report the exact shape of the objects presented. However, subjects did show repetition blindness when presented with the same stimuli but told ahead of time to name the objects

(monkey in each case) (see Figure 1; Bavelier, 1992, 1994). By showing that two different objects only compete for features that are required by the task, this work establishes that the nature of the information integrated in object-specific representations is a function of the task requirements.

We are also exploring whether parts of objects are being processed in the same way than objects. We have proposed that object representations are critical for guiding behavior. This property is believed to be specific to the level of object, and therefore not true for parts of objects. If so, the mechanism operating at the object level should not apply to their parts. We have found experimental evidence for this view, suggesting that the visual routines that apply to the construction of object representations differ from those involved in representing an object's parts (Bavelier, Deruelle and Proksch, Submitted).

Neural bases of Visual Selection and Visual Tracking of Objects

Another part of our work focuses on the neural systems which mediate the object-specific mechanisms we identified at the behavioral level. We have begun, using event related potentials, to study the time course of the neural activity associated with the building of object representations in memory. Our study indicates, for example, that object-specific representations are being established in memory as early as 200 ms after the initial presentation of the scene (see Figure 2; Bavelier, Barrera and Neville, 1995). Although this time is surprisingly short (it takes a minimum of 80 ms for an action potential to reach the infero-temporal cortex, the part of cortex responsible for early visual recognition), it fits well with recent reports that earlier stages of visual processing (visual categorization) can occur as early as 150ms (Thorpe et al., 1996).

The ERP technique has a remarkable temporal resolution but its spatial resolution, while improving, is still limited. Thus, we will complement our studies using functional MRI. This technique allows us to assess at a more detailed anatomical level the neural substrates involved in the processes we study.

Visual Selection/Visual Tracking of Objects and Brain Plasticity

There is considerable anecdotal evidence that in humans deprivation of input in one sensory modality leads to compensatory increases in the remaining abilities. Previous research on deaf individuals suggests they have enhanced high level visual functions, in particular those which require visual attention and memory. Thus, hearing and deaf subjects tend to have similar visual acuity and similar performance in simple visual discrimination task such as judging the direction of a single moving stimuli. However, deaf individuals are more accurate than matched hearing controls in more demanding tasks such as tracking an object which moves among other objects in the periphery of the visual field (Neville and Lawson, 1987).

Conclusions

In a collaborative study with Dr. H. Neville from U. Oregon and Dr. D. Corina from U. Washington, we are currently investigating the possible neural bases of such behavioral changes. The hypothesis we are currently pursuing proposes that the behavioral advantage shown in deaf individuals is due to the recruitment for visual processing of areas mediating auditory functions in hearing subjects (for example, the primary auditory cortex). The high spatial resolution of the fMRI technique gives us a unique opportunity to test for specific functional changes between deaf individuals and their controls within pre-specified areas (see Figures 3 and 4). This research will help characterize how the functional specialization of visual areas arises during normal development. The study of the effects of altered experience during human development is also critical for understanding the timing, pathogenesis and rehabilitation procedures best suited to compensate for congenital sensory deprivation.

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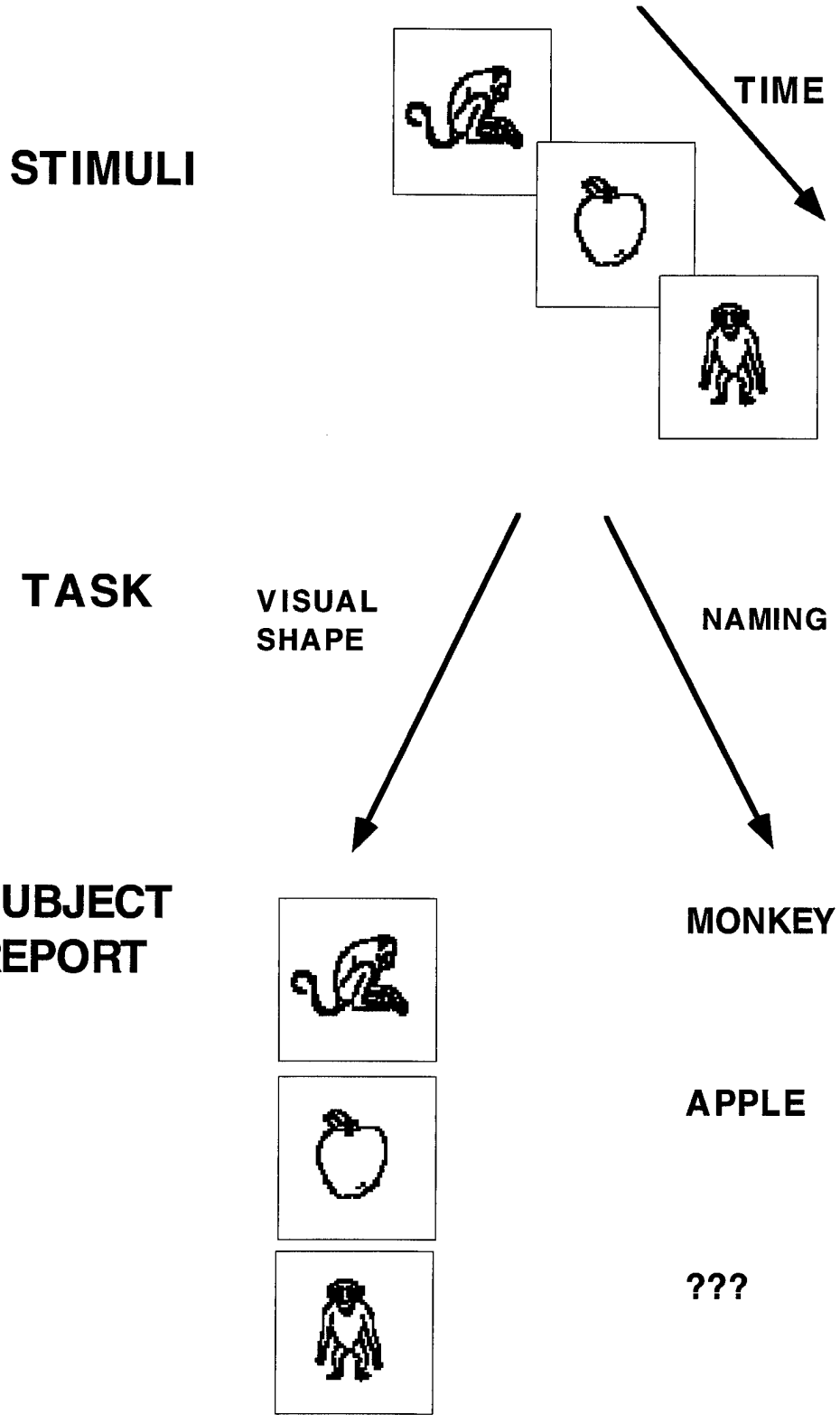

Figure 1: Example of a sequence of stimuli presented during a repetition blindness experiment. For the same stimuli, subjects did not show repetition blindness in the visual shape task but did show repetition blindness in the naming task.

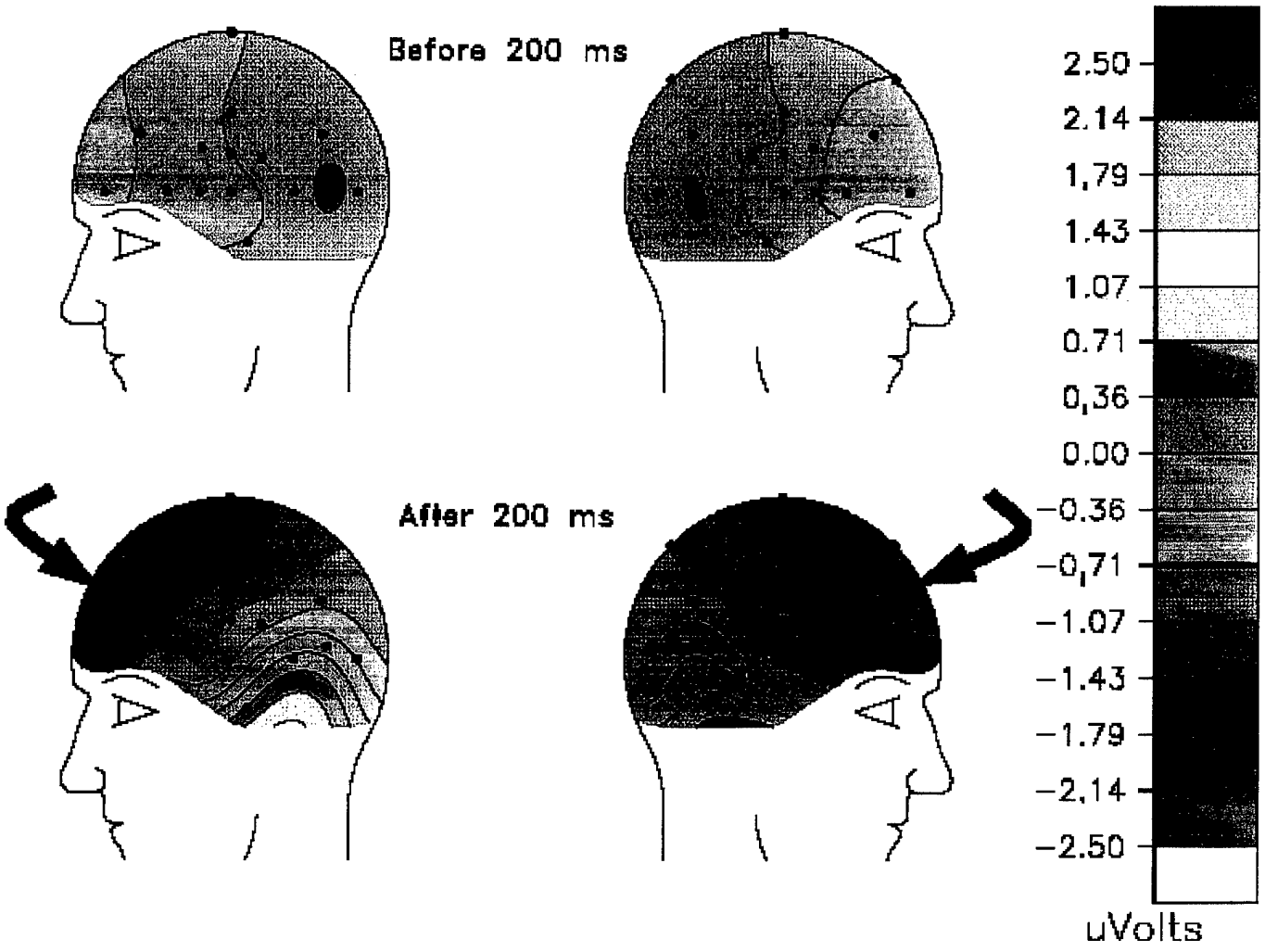

Figure 2: Setting up object-specific representations: Potential maps of the difference between successful and unsuccessful set-up before and after 200 ms. After 200 ms, a significant difference is observed.

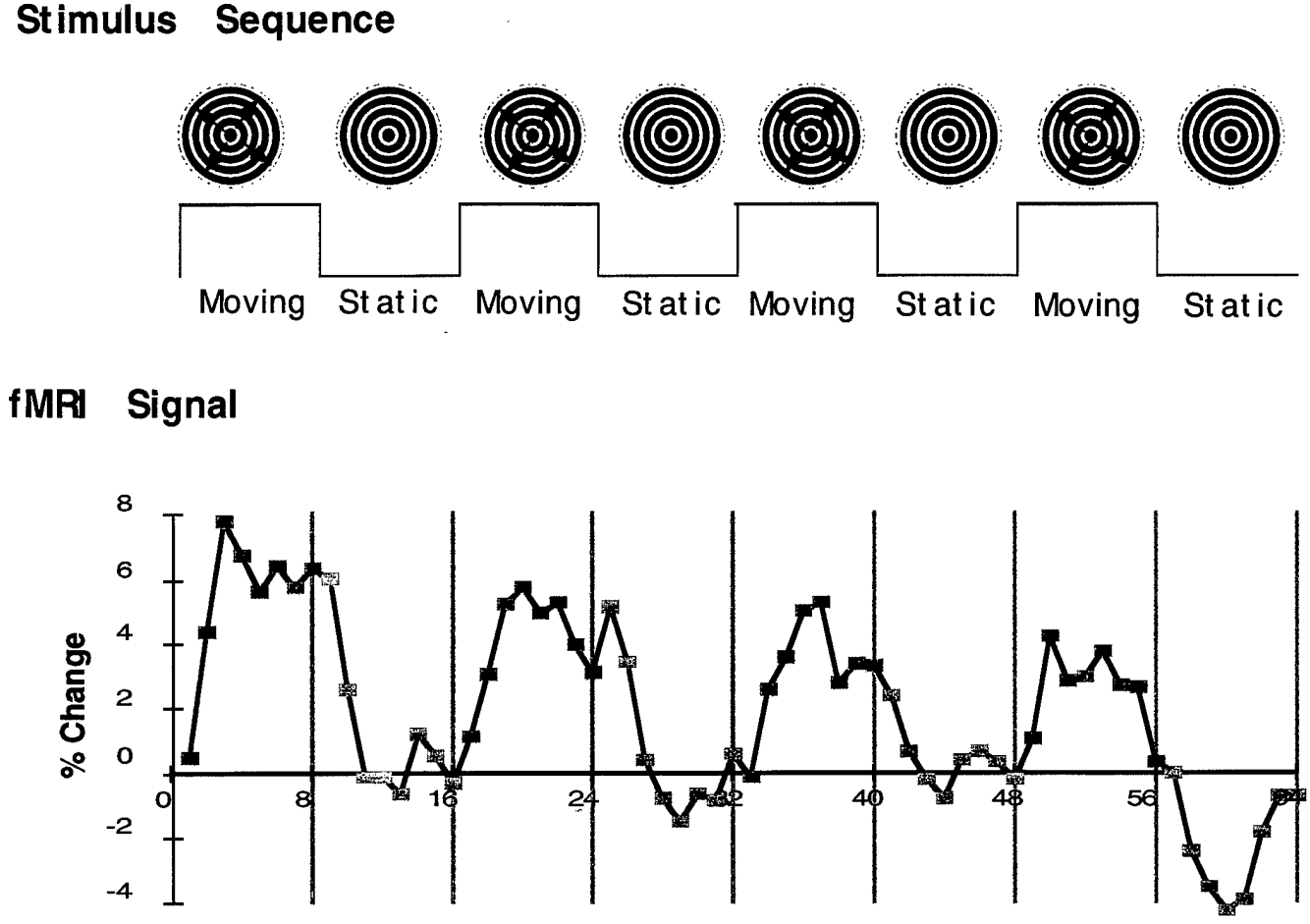

Figure 3: Example of the stimulus sequence used in a preliminary experiment investigating visual motion processing in deaf and control individuals. Active areas show a varying fMRI signal with the stimulus alternation.

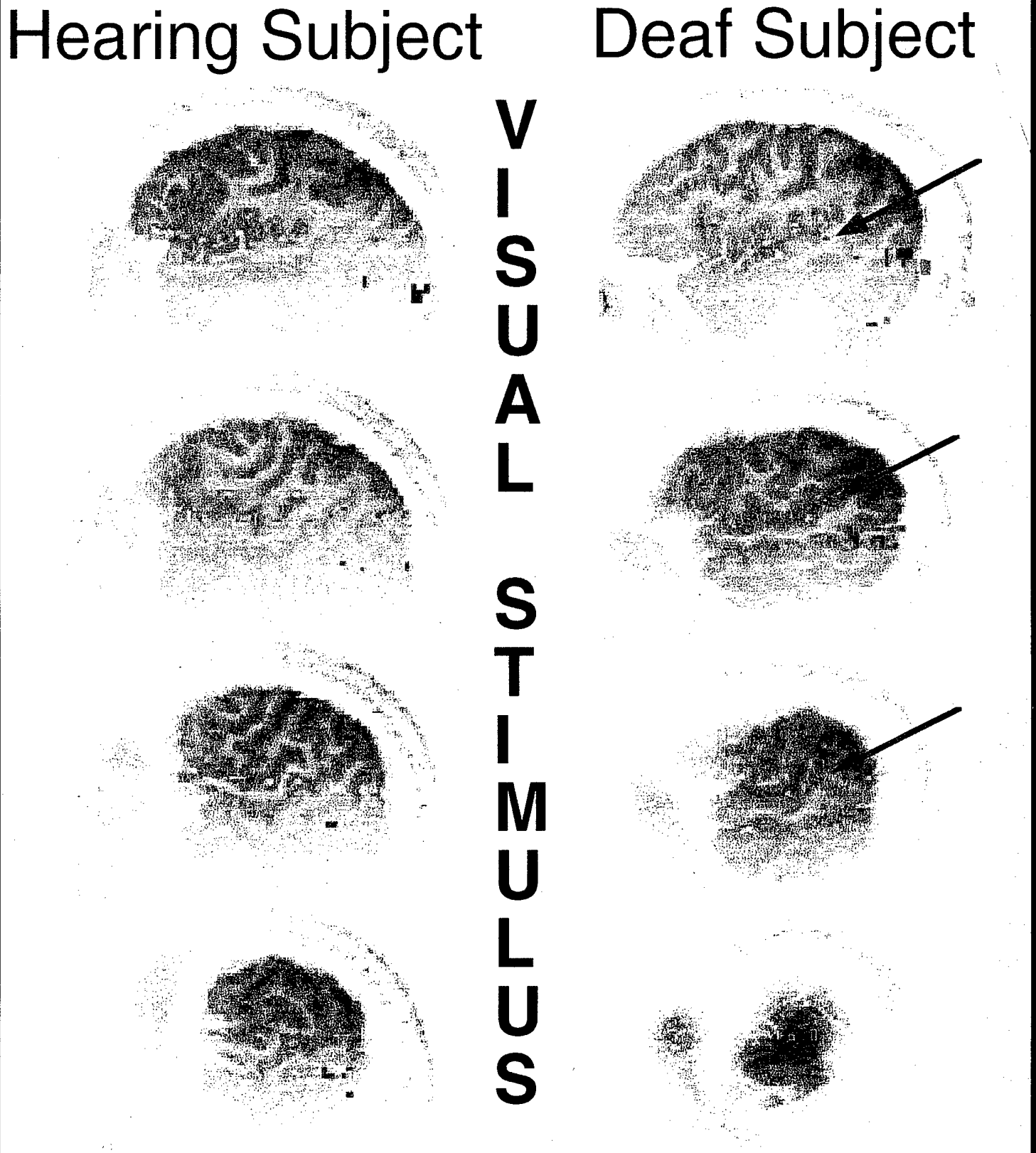

Figure 4: Pattern of activation for one deaf and one control hearing subject when processing moving stimuli. These preliminary data suggest activation, in deaf but not hearing controls, of areas that normally mediate auditory processing (see blue arrows).

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GUINEVERE EDEN, D.PHIL.

Functional MRI Studies of the Pathophysiology of Developmental Dyslexia

Introduction

Dyslexia is an impairment in reading that can result from an abnormal developmental process (developmental dyslexia) or cerebral insult (acquired dyslexia). Learning to read can become a serious obstacle for 4 to 10% of the children in elementary schools worldwide. It has long been known that the clinical manifestations of developmental dyslexia are varied. In addition to their reading difficulties, individuals with developmental dyslexia exhibit impairments in their ability to process the phonological features of written or spoken language. Recently, Dr. Eden and other investigators have demonstrated that these individuals are also impaired on a number of visual tasks, involving visuomotor, visuospatial and visual motion processing. These results suggest that the pathophysiology of dyslexia is more complex than originally thought, extending beyond the classically defined brain language areas.

Methods and Results

Dr. Eden's work at Oxford, UK and at the National Institute of Mental Health has led her to initiate a research program at Georgetown University to investigate the existence of a deficit in sensory information processing in dyslexia that is common to multiple sensory modalities. Unlike language, studies of human visual processing have had the benefit of a more detailed understanding of the anatomy and physiology of the visual system gained from experiments with non-human primates. From these studies, schemes for regional functional specialization of the visual cortical pathways have been described. Notable among these is a dichotomy based on relative selectivity of particular cortical areas for processing color and form versus motion. More recently functional neuroimaging utilizing positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have identified a specific motion sensitive area, V5 or MT, thought to be dominated by input from the magnocellular stream. In a recently published Nature article, Dr. Eden and colleagues reported on a motion processing deficit in the visual system of dyslexics. In those experiments (Figure 1), passive perception of visual motion in dyslexics failed to produce any detectable task-related functional activation in area V5/MT (part of the magnocellular system). In contrast, all control subjects had a robust response in the same region. They concluded that dyslexics suffer from abnormalities of the fast visual processing pathway (magnocellular), whilst the slower form processing system (parvocellular) was unaffected. This profound physiological abnormality was accompanied by a relatively subtle behavioral deficit in visual motion detection.

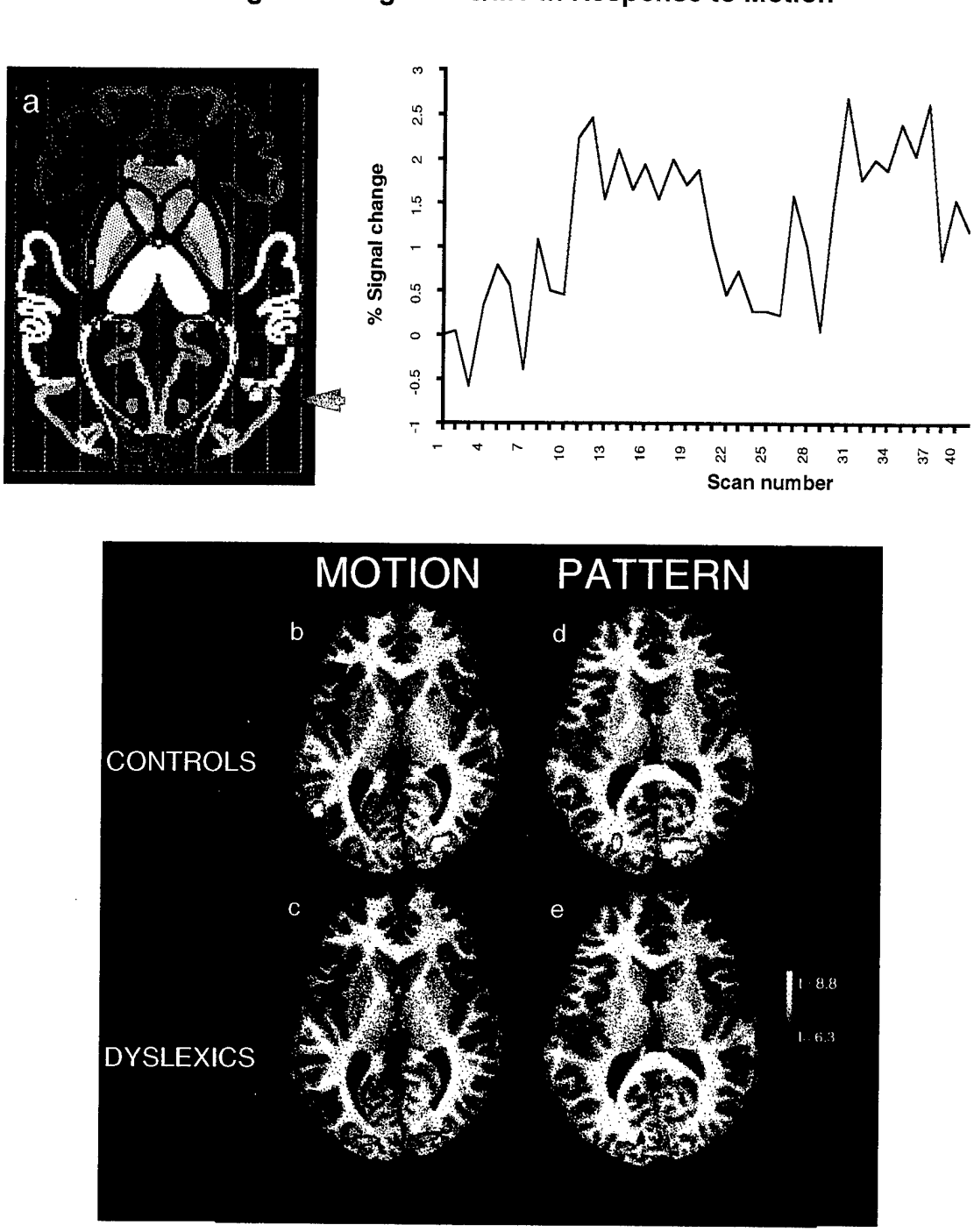

Figure 1: Top Panel: Example of a time series showing the response to motion of a single subject's most significantly activated single voxel in the area V5/MT.
Bottom Panel: Group analysis reveals that area V5/MT shows a task-related response to motion in the control group but not in the dyslexic group. The response is similar in both groups when viewing a pattern stimulus, demonstrating that the difference between the groups is stimulus specific.

Conclusions

Dr. Eden is currently extending these recent fMRI studies demonstrating differences in functional activity of brains of dyslexics to include the auditory system and to relate them to mechanisms underlying phonological processing. Behavioral and psychophysical testing are used to assess the range and distribution of language and sensory processing abilities in normal controls and individuals with dyslexia in an attempt to further understand the characteristics and heterogeneity of developmental language disorders. A 1.5 Tesla magnetic resonance imaging system is used to collect conventional structural brain images that can be subjected to morphometric analysis. Functional magnetic resonance imaging is employed for physiological measurements. Integration of the resulting behavioral, anatomical and physiological information into structure/function correlations is a principal goal of Dr. Eden's research program. Using these techniques she will examine the degree to which these physiological changes correlate with the observed behavioral deficits. Comparison of results across modalities will reveal to what degree dyslexics exhibit functional deficits common to vision and audition, and may suggest the neuroanatomical localization of a common neural substrate. The results of these experiments will provide new information concerning the neural substrates responsible for the visual, auditory and phonological abnormalities characteristic of developmental dyslexia. Moreover, the laboratory is interested in the development of new diagnostic tools that may allow earlier and more accurate identification of individuals with developmental language disorders.

DR. RHONDA FRIEDMAN, PH.D.

Dr. Friedman's research encompasses three major projects: (1) Written language processing in Alzheimer's Disease; (2) Cognitively-based treatments of acquired dyslexias; and (3) Evaluating cognitive neuropsychological models of language recovery with fMRI.

Project 1: Written language processing in Alzheimer's Disease

The project focusing on language deficits in patients with Alzheimer's Disease has yielded two sets of interesting results. One concerns semantic processing; the second deals with the reading of pronounceable nonwords.

1. Patterns of Lexical and Semantic Associative Priming in AD

The purpose of this ongoing study is to examine the underlying basis of the semantic processing deficits that are a major component of the language deficit of Alzheimer's Disease. There are two competing hypotheses. The first claims that concepts themselves are degraded in semantic memory. The second claims that concepts remain intact, but access to the concepts is impeded.

One source of support for the notion of degraded representations comes from studies indicating that more general information, such as knowledge of superordinate category, is better preserved than more specific features of objects. In support of the impeded access hypothesis are studies of semantic priming, which demonstrate that AD patients show normal facilitation of their response times in oral reading and lexical decision tasks if the target word is preceded by a semantically-related prime word.

In previous work, we questioned the inferences that could be drawn on the basis of results from priming studies. The conclusion that normal priming indicates intact semantic networks is based on the assumption that the relationships that can be shown to exist between words reveal the relations that hold between the meanings or concepts underlying these words. We posited that the priming of associative relationships between words may be distinct from the priming of semantic relationships between concepts. In typical priming studies, these relationships are confounded (the word pair dog-cat is both semantically and associatively related).

Our current study employed a priming task in which the nature of the relationships between the prime and target are crucial. Tallies from a word association task were used to generate four types of word pairs: 1) subordinate exemplar: nonassociated superordinate category (e.g., daughter: relative); 2) two nonassociated exemplars of the same category (e.g., square: triangle); 3) lexical associates that have no

semantic relationship (e.g., rags: riches); and 4) word pairs that are unrelated semantically and associatively (e.g., uncle: metal).

Subjects to date include 16 healthy elderly controls and 5 probably AD patients with mild dementia. The results are presented in Table 1. The normal controls showed a priming effect for all three of the experimental conditions: subordinate-superordinate pairs, coordinate pairs, and lexically associated pairs. In contrast, the AD patients showed priming for only two of the conditions, failing to demonstrate priming effects for the non-associated coordinate pairs. That is, patients showed no spread of activation between semantically related coordinates in memory that are not strong lexical associates, though age matched healthy controls did show priming of these relationships.

These results are consistent with the notion that priming effects may reflect strong associations between words, while not necessarily speaking to the issue of conceptual relatedness. Therefore, previous studies invoking priming effects in AD as evidence of intact semantic networks may be invalid. Relationships between concepts, as tested in our paradigm, do appear to be impaired in patients with AD. Further, our finding that for nonassociated pairs, only superordinate but not coordinate relationships facilitate responses in AD patients, is consistent with the notion that specific semantic features may be lost before more global semantic features in early AD.

Table 1
Number of Correct Responses (out of 25) Threshold Priming Task

	Priming Condition	Control Condition
NORMAL CONTROLS		
Associate	16.3 ****	11.5
Superordinate	15.8 ***	12.6
Coordinate	14.0 ***	11.0
Nonrelated	11.2	10.6
AD PATIENTS		
Associate	14.2 *	10.2
Superordinate	14.0**	10.6
Coordinate	12.2	11.2
Nonrelated	8.2	10.6

* p=.06 ** p<.05 *** p<.01 **** p<.001

2. The Reading of Nonwords by Patients with AD

Previous work (Friedman et al, 1992) had revealed that, compared with age-matched normal controls, patients with AD demonstrated disproportionate difficulties reading nonwords that have no real word analogies in the English language (e.g. bwiv). These results were interpreted to indicate that AD patients' oral reading was accomplished through a lexical mechanism, the same mechanism that is ordinarily employed by normal controls to read nonwords. As many of the nonwords with no analogies used in that study were both orthographically and phonologically unfamiliar (i.e. without analogies), it could not be determined whether the lexicality effect of nonword reading was due to familiarity of orthographic forms or phonological forms or both.

To address this issue, in a study conducted this past year, orthographic familiarity was varied between the different nonword oral reading conditions. Half of the nonwords presented for oral reading were orthographically analogous to real words (e.g. nart) while half contained unusual letter combinations (e.g. hilx), but all nonwords were composed of phonological forms that do have real word analogues in English. These nonwords were presented one at a time on a computer screen for oral reading. Subjects were 18 patients with probable AD and 17 elderly controls. The results can be seen in Table 2. Statistical analyses revealed a significant group difference and a significant effect of stimulus type, but no interaction. This contrasts with the results of a repetition task, in which phonologically unusual nonwords were contrasted with phonologically regular nonwords. A significant interaction between subject group and nonword type was found for the repetition task. (See Table 2.) The results support the conclusion that lexical effects in nonword reading occur at the level of phonological processing, which is impaired in AD patients, while orthographic processing remains intact.

Table 2
Proportion of Nonwords Read and Repeated Correctly

	AD Patients	Normal Controls
READING		
Orthographically Regular	.56	.92
Orthographically Unusual	.40	.79
REPETITION		
Phonologically Regular	.74	.95
Phonologically Unusual	.37	.71

Project 2: Cognitively-Based Treatments of Acquired Dyslexias

The purpose of this project is the development of a set of therapy programs that are shown to be effective in the treatment of acquired disorders of reading (acquired dyslexias, also known as alexias). This goal is achieved through the development, implementation, and evalulation of several experimental therapies, each targeted for a specific type of reading deficit, based upon a cognitive neuropsychological model of reading. The data obtained from this study are also used to improve our models of normal reading, which may lead to more effective methods of teaching reading to both normal and developmentally dyslexic children.

Patients with acquired reading disorders following stroke are referred to our project by neurologists or speech pathologists for further evaluation. The patient's reading and other cognitive skills are assessed using a battery of screening tests that we have developed over the past several years. Based upon the results of these tests, the patient's alexic disorder is characterized. Patients whose deficits are among those that are the focus of this project are assigned to one of several possible treatment programs devised specifically for that type of deficit enables us to consider a key issue in rehabilitation research, whether it is more efficacious to attempt to restore a loss cognitive processing mechanism or to by-pass the impaired process and substitute another means of achieving the intended goal. Additionally, factors that might predict the success of a particular treatment for a particular patient are examined. (See the section on fMRI studies.) The overall structure of this study consists of single case studies, replicated over several patients, each employing a multiple baseline design.

The following results have been obtained over the past year.

1. Pure Alexia

A. Semantic Therapy. The theoretical basis for this therapy was the suggestion in the literature that some information is passed on to the semantic system when pure alexic patients view words that they fail to identify. The task here, then, focuses on semantics: the patients say "yes" if a rapidly presented word belongs to a given category or "no" if it does not belong.

Two of the three patients entered into this treatment successfully learned to categorize the trained words. Generalization to untrained words was minimal. However, length effects remained, and no part of speech effect emerged, contrary to what was expected for semantic reading. It appeared, then, that whatever was being learned might not be a semantic reading strategy. To pursue this further, the task was switched to oral reading, i.e. the patient simply read each rapidly presented word aloud. Both patients who had been successful with the categorization task were also successful with the oral reading task; the third patient, DL, failed to reach criterion on both tasks, after 12 sessions on the categorization task and 17 sessions with the oral reading task. In order to replicate our prior results, a new patient, SV, has been evaluated, and has just begun this therapy.

To further rule out reading via semantics as the reason for the two successful patients' improvement in reading trained words, functor words, which are relatively low in semantic value, and pseudowords, which have no semantic value, were substituted for the nouns in the oral reading task. Both patients

improved their reading of the trained functors and pseudowords (See tables 3 and 4 and Figures 1 and 2).

This study has succeeded in demonstrating that for some patients with pure alexia, improvement may be effected by reteaching the patient to rapidly recognize words that had been known previously, but can no longer be recognized rapidly. This type of relearning does not appear to generalize to items that are not taught.

Table 3. *FT's performance on Functor Words*

	Pre-Treatment	Post-Treatment
Trained Words	66%	98%
Untrained Words	55%	53%

Table 4. *RS's performance on Functor Words*

	Pre-Treatment	Post-Treatment
Trained Words	77%	95%
Untrained Words	67%	80%

Figure 1:.

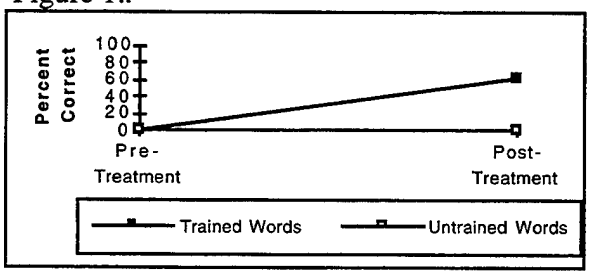

Figure 2:

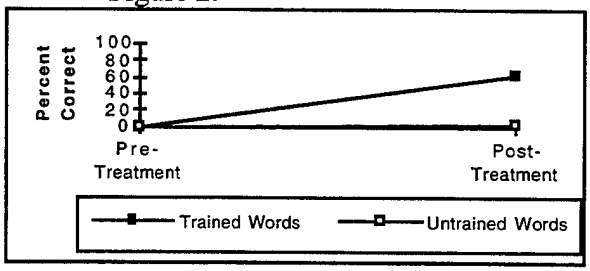

Figure 1: Patient FT's accuracy in reading trained and untrained nonwords, before and after rapidly-presented word reading treatment.

Figure 2: Patient RS's accuracy in reading trained and untrained nonwords, before and after rapidly-presented word reading treatment.

B. Speeded Letter-by-Letter Treatment. In this treatment, patients are first trained to use a tactile/kinesthetic method to facilitate accurate letter naming, and are then trained to read words in letter-by-letter fashion while receiving feedback regarding their response time. Patient DL, who did not improve following semantic treatment, was able to not only improve his reading accuracy, but to decrease his reading time as well (see Figure 3). These results indicate that restoration of function may, in some cases, be achieved by engaging another modality of stimulus input.

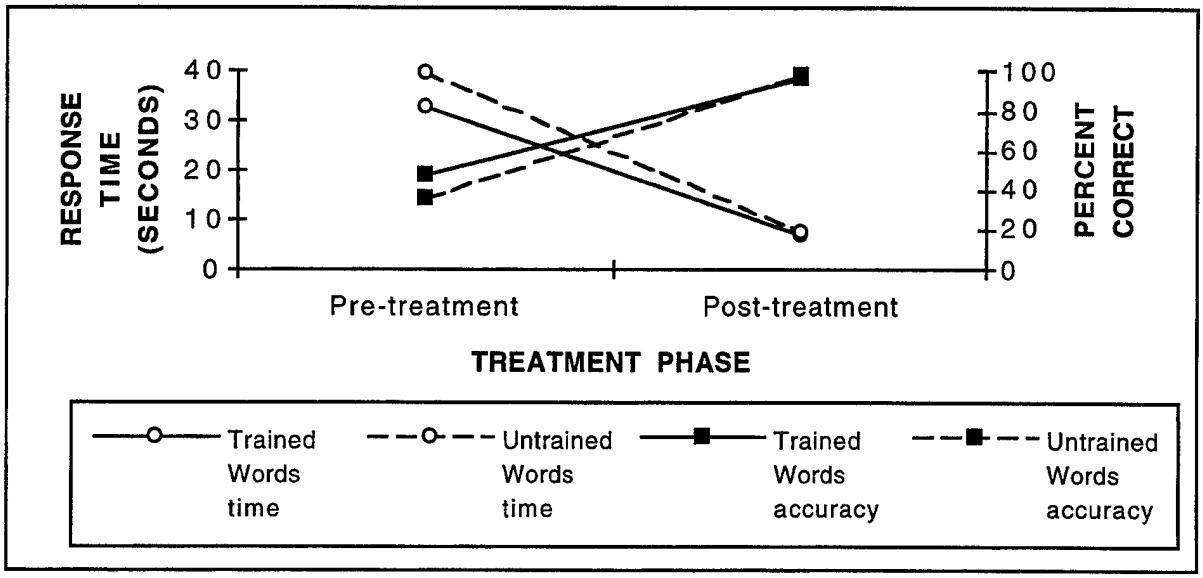

Figure 3: Patient DL's accuracy and speed of reading trained and untrained words, before and after tactile-kinesthetic plus rapid letter naming treatment.

C. Hybrid Treatment. Hybrid treatment combines the successful elements of the two approaches discussed above, i.e. rapid word presentation and speeded letter-by-letter reading. The patient is trained to (1) rapidly recognize a set of the most frequently occurring words, (2) quickly read less frequently occurring words in letter-by-letter fashion, and finally (3) to combine using both strategies when reading sentences. It was created during the past year, and patient FT has just completed the first phase (See table 5).

Table 5. *FT's Hybrid Sight Word Reading*

Pre-Treatment	Post-Treatment
65%	85%

2. Phonological/Deep Alexia

Patients with this type of alexia cannot read via a system of assembled phonology (letter - sound conversion). Thus unfamiliar words and pronounceable nonwords are read poorly relative to real familiar words.

A. Bigraph-Phoneme Conversion Treatment. This therapy is predicated on the premise that grapheme-phoneme conversion is not the most natural way to translate letters into sounds. Our treatment uses the bigraph syllable, which is a more natural and psychologically real unit of language. In this treatment, patients are trained to pronounce syllables by pairing them with key words (e.g. li - lips), and then to sound out words by combining these syllables. The success of this treatment to date confirms our prediction. (See Figures 4-6).

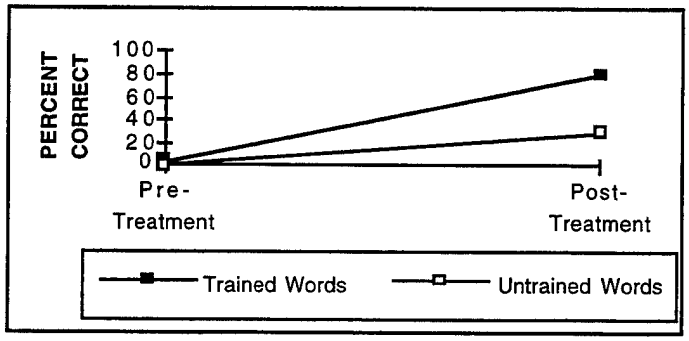

Figure 4: Patient LR's accuracy in reading trained and untrained words, before and after bigraph syllable treatment; Set 1 words.

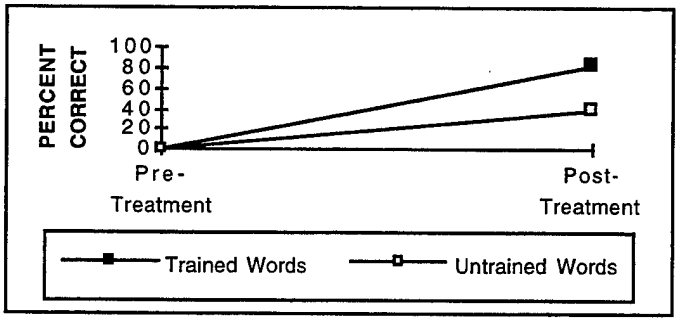

Figure 5: Patient LR's accuracy in reading trained and untrained words, before and after bigraph syllable treatment; Set 2 words.

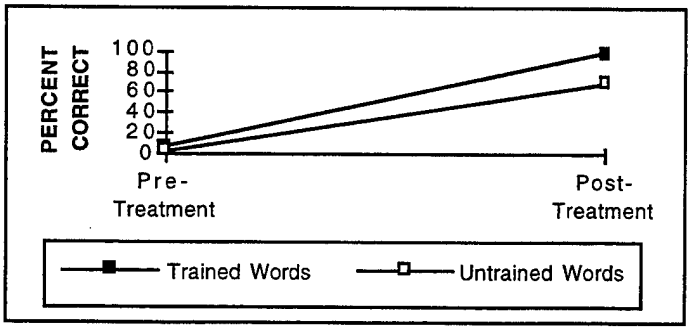

Figure 6: Patient KT's accuracy in reading trained and untrained words, before and after bigraph syllable treatment; Set 1 words.

B. Phonological Neighborhood Treatment. This therapy derives from connectionist models of word reading, which hold that the ability to read unfamiliar words is related to the strengths of the connections between the orthographic and phonologic units of which they are composed. These strengths are related to the number of times that words containing those connections have been read, i.e. the number of times that the orthography has been paired with the phonology. In the therapy, the oral reading of a group of orthographically similar words is trained to criterion, then the patient is tested on his/her ability to read a target word with overlapping orthography. (Example: came, case, cape, bake, make, lake; target cake) The models predict that the repeated pairing of each of these written words with their pronunciations should strengthen all of the component orthography to phonology connections needed to orally read the target word.

Patient GR read target words of Set 1 with an average pre-treatment accuracy of 27%. After training, she succeeded in reading four out of the five target words with greater than 90% accuracy, while

performance on most of the control words (words not composed of trained neighborhoods) remained poor. These results support the notion that connection strengths between components of cognitive processing can be reestablished following brain injury.

C. Semantic Mediation Treatment. While the previous therapy concentrated on "restoring" the damaged route to reading, this therapy takes the "re-organization" approach. It attempts to use the semantic route to circumvent the problems in the phonological reading route. This treatment pairs difficult words low in semantic value (functors and verbs), which are difficult for these patients to read, with homophones (words that are pronounced the same) or near homophones (e.g. in and inn, me and meat) that have high semantic value. This therapy was created during the last year. A new patient, DN, has been evaluated and has just initiated this treatment.

Project 3: Evaluating Neuropsychological Models of Language Recovery with fMRI

Several fMRI studies are underway to (1) determine the potential usefulness of the technique of fMRI in predicting the outcomes of various treatments for certain types of language and reading disorders; (2) elucidate the mechanisms of recovery from aphasic and alexic symptoms; and (3) provide data that will help refine current neuropsychological models of language and reading.

The first part of this past year was spent obtaining the appropriate equipment for stimulus presentation, getting it in working order, and adapting it for our needs. We have begun to run studies on normal control subjects.

One practical issue that we are investigating is the role of stimulus presentation rate upon the amount of detectable fMRI activation. It was hypothesized that if stimuli are presented too rapidly for adequate stimulus processing, activation may not be seen. On the flip side, if the delay between stimuli is too long, subjects may finish processing the current stimulus and proceed to think about other things while waiting for the next stimulus to appear. This situation might also result in unreliable data. To explore these possibilities, we presented stimuli to normal control subjects under three conditions: 1. All letter strings were presented for 200 msec. 2. All letter strings were presented for 500 msec. 3. All letter strings were presented for 1000 mec. For all subjects studied thus far, the 500 msec condition clearly produced the greatest activation levels, and for some subjects, activation was only seen in the 500 msec. condition.

As a first step in localizing the activation that might occur during single-word reading and to establish the utility of the fMRI technique for studying the components of reading, we compared the activation obtained when looking at pseudowords with the activation obtained when looking at a centrally located fixation dot. We expected that the visual processing involved in looking at pseudowords would be different from that involved in looking at a static fixation dot, especially with pseudowords presented at a rate of 2 per second and the fixation dot continuously displayed on the screen without changing. As expected, the activation in the occipital regions was more extensive when viewing pseudowords (Figure 1A) than when viewing the fixation dot (Figure 1B). This finding that a rapidly changing visual-verbal stimulus produces greater occipital activation than a static nonverbal visual stimulus confirms that the fMRI technique as we are using it can meaningfully delineate regions of brain activity as a function of different types of visual stimulation and processing.

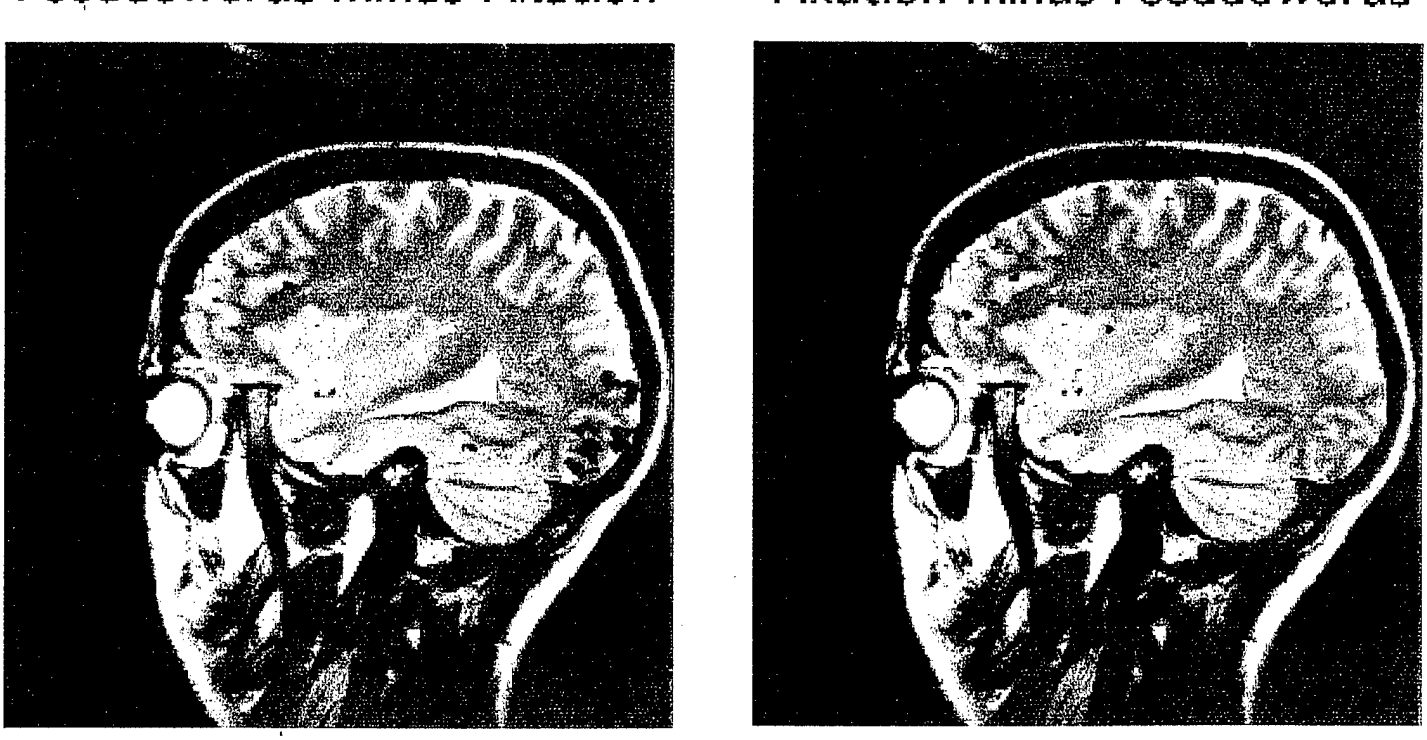

Figure 7: Activation on fMRI scan from a single subject viewing pseudowords compared with viewing a single fixation dot.

Figure 7A: pseudowords minus fixation dot.

Figure 7B: fixation dot minus pseudowords.

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Friedman, R. B., Ferguson, S., Robinson, S., and Sunderland, T. Dissociation of mechanisms of reading in Alzheimer's Disease. Brain and Language (1992) 43:400-413.

MICHAEL ULLMAN, PH.D.

Introduction

Dr. Ullman's research investigates the neural bases of language. He has been addressing this issue by attempting to answer four questions: 1) How many neural systems underlie language? 2) What functions does each carry out? 3) Are these system(s) localizable, and if so, to where? 4) What neural mechanisms underlie the computations of these functions?

His current research (Ullman et al, in press) focuses on applying these questions to the two fundamental capacities of language: The mental lexicon, which contains stored information about the sounds and meanings of words; and the mental grammar, which contains generative rules which combine words into larger words, phrases and sentences.

Previously, two competing theoretical frameworks have attempted to answer these questions. One has proposed that there are distinct brain regions, one specialized and dedicated to the mental lexicon (left temporal regions), the other specialized and dedicated to the mental grammar (left frontal regions, including Broca's area). The second theoretical framework has contested this dichotomy, and has proposed that there is a single brain system underlying both the lexicon and grammar, as well other non-language functions.

Dr. Ullman has been investigating a third alternative: That there are indeed distinct brain systems subserving lexical memory and grammatical rules, and that each of these systems is indeed specialized for a different type of computation, but that neither system is dedicated to language; rather each system is specialized for a class of computations, and subserves a wide variety of language and non-language functions.

Specifically, Dr. Ullman and his colleagues have been testing the hypothesis that 1) Lexical memory is part of declarative memory, a well-studied brain-systems construct previously implicated in the memory of facts and events, and largely localizable to temporal-parietal/medial-temporal regions; and 2) Grammatical rules are processed by the procedural system, a distinct brain-systems construct previously implicated in the learning and processing of motor and cognitive skills, such as riding a bicycle or skilled game playing, and at least partly localizable to frontal cortex and the basal ganglia.

To test this hypothesis, Ullman devised a task based on a simple linguistic system in which reliance on grammar and lexicon differs, while other factors are held constant. Regular (look-looked) and irregular (dig-dug) past tense forms of verbs are well-matched in complexity (one word), syntax (tensed), and meaning (past). But regular verbs are predictable in form (verb stem + -ed), and new ones are constantly being added to the language (faxed, moshed), whereas irregular verbs are unpredictable (compare sing-sang, fling-flung, bring-brought), and constitute a fixed list. A simple theory is that regular forms are generated by a rule, and irregular forms are memorized. A number of studies from psycholinguistics and language acquisition support this theory.

If indeed (a) irregulars are stored words, (b) the temporal-parietal/medial-temporal declarative system underlies word memory, (c) regulars are rule-products, and (d) the frontal/basal-ganglia procedural system underlies the processing of rules, then the following double dissociations are predicted. Patients with impairments of lexical, or more generally, declarative memory, from damage to temporal or parietal cortex, should be worse at producing past tense forms of irregulars (dig-dug) than regulars (look-looked) or novel verbs (plag-plagged). In contrast, patients with impairments of rules, or of procedures in general, from damage to frontal cortex or the basal ganglia, should be worse at producing the pasts of regular or novel verbs than of irregular verbs.

Methods

A past tense production task was given to patients and control subjects, who read aloud sentence pairs, filling in the blank(e.g., "Every day I dig a hole. Just like every day, yesterday I _____ a hole."). Twenty sentence pairs contained irregular verbs, 20 contained regular verbs, and 20 contained novel verbs. To measure lexical memory, subjects were asked to name 84 drawings of objects. To measure memory for facts and events, subjects were asked to retrieve information about facts and events.

Impairments of Lexical Memory

Alzheimer's Disease: Existing evidence suggests that patients with Alzheimer's disease (AD) have impairments learning new and remembering old facts, events, and words. In contrast, they have a relative sparing of motor and cognitive skills, and of sentence processing. Their neuropathology shows a similarly intriguing dissociation: there are high densities of neurofibrillary tangles in temporal and temporo-parietal regions, and low densities in the basal ganglia and in frontal regions, including Broca's area. If declarative memory also subserves the lexicon, while the procedural system also subserves grammatical rule processing, then memory impairments in AD should be associated with impairments producing irregulars (dug), but not with impairments producing regulars (looked) or novel verbs (plagged).

Indeed, Ullman and his colleagues found that across the 24 AD patients they tested, performance at object naming correlated significantly with performance at producing irregulars ($p<.005$), but not regulars ($p=.118$) or novel verbs ($p=.152$). Similarly, performance at fact retrieval correlated significantly with performance at producing irregulars ($p<.005$), but not regulars ($p=.072$) or novel verbs ($p=.107$). Moreover, the five most anomic AD patients (those with the worst object naming scores) were significantly worse at producing irregulars than regulars ($p<.005$) or novel verbs ($p=.016$).

Posterior (Wernicke's) Aphasia is associated with stroke-lesions to temporal or temporo-parietal areas, and difficulties with word-finding, but not with grammatical processing. Five posterior aphasics were also tested. They showed the same pattern as the anomic ADs, being worse at producing irregulars than regulars ($p=.024$).

Impairments of Grammatical Rules

Parkinson's Disease: To demonstrate a double dissociation, Ullman turned to Parkinson's disease (PD), which is associated with the degeneration of dopaminergic neurons in the basal ganglia, causing excess

inhibition of motor and other frontal cortical areas to which the basal ganglia project. This is thought to explain the suppressed movement (hypokinesia) characteristic of PD, and may also explain observations that PD patients have impairments in the learning of motor and cognitive skills, and in the processing of sentences. Thus if the left basal ganglia project not only to left frontal motor areas, which control right-side movement, but also left frontal areas involved in grammatical rule processing, then left basal ganglia degeneration leading to right-side hypokinesia may also lead to rule suppression (problems producing pasts of regular and novel verbs), with relative sparing of the production of irregular pasts.

Testing of 28 PD patients revealed that, across patients, right-side hypokinesia correlated significantly with impairments producing regulars ($p<.005$) and novel verbs ($p<.005$), but not irregulars ($p=.161$), even when left-side hypokinesia was partialed out. However, left-side hypokinesia did not correlate with impairments in the production of any of the three verb types. This contrast underscores the role of the left basal ganglia in rule processing. Moreover, the five most hypokinetic PD patients were significantly worse at producing regulars ($p=.013$) and novel verbs ($p=.016$) than irregulars.

Anterior (Broca's) Aphasia is associated with stroke-lesions to Broca's area and adjacent left frontal perisylvian cortex, plus underlying white matter and the basal ganglia, and is characterized by "agrammatism" (omission or misuse of grammatical morphemes, and difficulty understanding sentences) and articulation problems, while access to content words such as verbs and nouns is often relatively spared. Five anterior aphasics were also tested. They showed the same pattern as the hypokinetic PDs, being worse at reading regular than irregular past tense forms ($p<.005$).

A Role for the Basal Ganglia in Grammatical Rule Processing

Huntington's Disease: In PD patients, the suppression of motor programming (caused by basal ganglia degeneration leading to the inhibition of frontal cortical areas) was associated with a suppression of rule programming. A complementary demonstration of a role for the basal ganglia in rule programming comes from Huntington's Disease (HD). Like PD, HD is accompanied by a loss of neurons in the basal ganglia (caudate and putamen), but, unlike PD, it is often in the inhibitory "indirect" pathway. This causes excess excitation in motor and other frontal cortical areas receiving basal ganglia projections, which is thought to explain why HD patients have unsuppressible movements (chorea, a type of hyperkinesia). Degeneration leading to such excess movements may therefore also lead to excess rule use.

Testing of 17 HD patients revealed errors which suggested excess rule use. They over-regularized (digged, dugged) at high rates, and produced two kinds of unusual errors, primarily on regular and novel verbs: multiply-suffixed forms (lookeded) and syllabically-suffixed forms (look-id). They did not produce analogous forms for irregulars (keptit, kep-it). Moreover, across subjects, chorea correlated with the rate of producing multiply- and syllabically-suffixed forms for regular and novel verbs ($p=.009$) and with the rate of over-regularization ($p=.047$). Thus chorea in HD is associated with overactive rule use. This suggests a role for the basal ganglia in rule programming.

Conclusions

In sum, patients with relative damage to temporal or parietal neocortex, and with general impairments of declarative memory (in Alzheimer's disease) or specifically of lexical memory (in posterior aphasia), had more trouble converting irregular verbs to their past tense forms than regular or novel verbs. Patients with relative damage to the frontal/basal-ganglia system, and with general impairments of procedures (in Parkinson's disease) or specifically of grammar (in anterior aphasia), showed the opposite pattern. These results support psycholinguistic theories that emphasize grammar and lexicon as distinct components over those that minimize or eliminate either, especially in the treatment of regular and irregular linguistic phenomena. Moreover, the findings extend the distinction between the temporal-parietal/medial-temporal declarative memory system and the frontal/basal-ganglia procedural system to the two major components of human language. Finally, one kind of basal ganglia lesion, which leads to the suppression of motor activity (in Parkinsons's disease), also led to the suppression of rule use. In contrast, another kind of basal ganglia lesion, which leads to excess motor activity (in Huntington's disease), also led to excess rule use. This suggests that basal ganglia circuitry contributes to grammatical rule processing in conjunction with frontal cortex, and implies that the well-studied basal ganglia circuits underlying motor programming may play a comparable role in rule programming.

Future Directions

Dr. Ullman's ongoing research is extending these findings in several directions. First, his new laboratory at GICCS is testing Italian AD, PD and aphasic patients in the production of regular and irregular Italian forms, with the purpose of investigating whether the findings in English can be generalized to other languages. Italian is particularly well-suited to this purpose because it has a very rich inflectional (conjugation) system, and has been very well-studied. This research is being carried out in collaboration with Stefano Cappa of the San Raffaele Institute in Milan. Second, his lab is testing patients with lesions or degeneration of the cerebellum. This study is motivated by independent evidence implicating the cerebellum in procedural memory. The research is being carried out in collaboration with Jeremy Schahmann of Massachusetts General Hospital (MGH) and Hilary Bromberg of Harvard University. Third, in a collaboration with Robert Savoy and Kathy O'Craven of the MGH-NMR center, Dr. Ullman's lab is carrying out a functional magnetic resonance imaging experiment, testing normal adults on their production of regular and irregular past tense forms in English.

References

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ANIMAL COGNITIVE NEUROSCIENCE: There are four faculty members who are grouped into this area. Dr. Kanwal investigates the processing of communication sounds and modeling of a neural network for multidimensional analysis of speech sounds and pattern recognition. Dr. Pekar utilizes magnetic resonance imaging (MRI) to evaluate brain structure, physiology, and function. Dr. Rauschecker examines the functional organization and plasticity of the cerebral cortex with an emphasis on the auditory cortex and multi-modal processing using single-neuron microelectrode recording, optical recording, and functional MRI. Dr. Wu studies large-scale neuronal events and distributed processes in the CNS.

JAGMEET KANWAL, PH.D.

Cortical organization and processing for acoustic communication

Introduction

Dr.Kanwal's research focuses on the cognitive and computational aspects of acoustic communication in mammals. Over the last six months his group has been actively working towards establishing a neurophysiology laboratory for conducting experiments on systems physiology and behavior. The primary focus of this laboratory will be to study the processing and representation of species-specific sounds in the mammalian auditory cortex. Most of Dr. Kanwal's prior research has focused on the mustached bat, *Pteronotus parnellii* whose auditory cortex is perhaps the best studied among mammals. For several reasons, the auditory system of bats is an excellent model to study neural mechanisms that may underlie processing of communication sounds in mammals, including speech sounds in humans. Humans have the unique and wonderful ability of verbal communication and yet we know little about its neural basis. For this type of communication, complex streams of speech sounds must be processed rapidly by the brain. At an elementary level, this corresponds to the processing of communication sounds in other mammals and perhaps originates from it. For example, recent studies suggest that the bat's cortex shows hemispheric asymmetry for processing communication sounds. In addition, combination-sensitive neurons and multidimensional representational schemes for processing complex syllables in the auditory cortex may be applicable to speech sound processing as well. Not surprisingly, the field of communication sound processing is re-emerging as an actively growing field as it attempts to understand the neural basis of speech perception and its commonalties with the audio-vocal system of mammals at the single neuron level. Bats are considered useful models because they employ a complex repertoire of sounds for acoustic communication rivaling that of the most vocal primate species. Even though the auditory system of microchiropteran bats is the best studied among mammals, previous neurophysiological studies on audition in bats have mostly focused on their echolocation behavior. Data obtained from these studies in conjunction with the results of experiments on communication sound processing will allow Dr. Kanwal's group to explore novel mechanisms and formulate new principles of auditory processing, especially at the cortical level. The goal is to use a neuroethological approach that combines systems physiology and behavior with computational experiments. New methods to be developed include telemetry and multi-site single unit recording from awake bats and, in collaboration with other faculty, optical and/or functional MRI imaging of cortical activity.

During the period of laboratory renovation and ordering of equipment, Dr. Kanwal availed the offer to join the group of Professor Neuweiler in Munich, Germany to initiate communication related neurophysiological research in a different species of bats. His stay in Germany for several months was supported by the Humboldt fellowship. During this time he successfully recorded from the cortex of the false vampire bat, *Megaderma lyra*, that forages using both echolocation and passive listening mechanisms. Figure 1 shows the amplitude oscillograms of a set of 14 stimuli that represent different types of calls produced by this species. Figure 2 shows the spectrograms of the same sounds. The bat calls were recorded, categorized and digitized using the "SIGNAL" program. The categorization is based on behavioral analysis in collaboration with Prof. Neuweiler's group. The arrangement of stimulus presentation is based on the structural features of these sounds, e.g., constant frequency, frequency modulation and noise bursts. The latter two are further subdivided into narrowband and broadband sounds. These data clearly show the wide variety of communication sounds produced by this species some of which

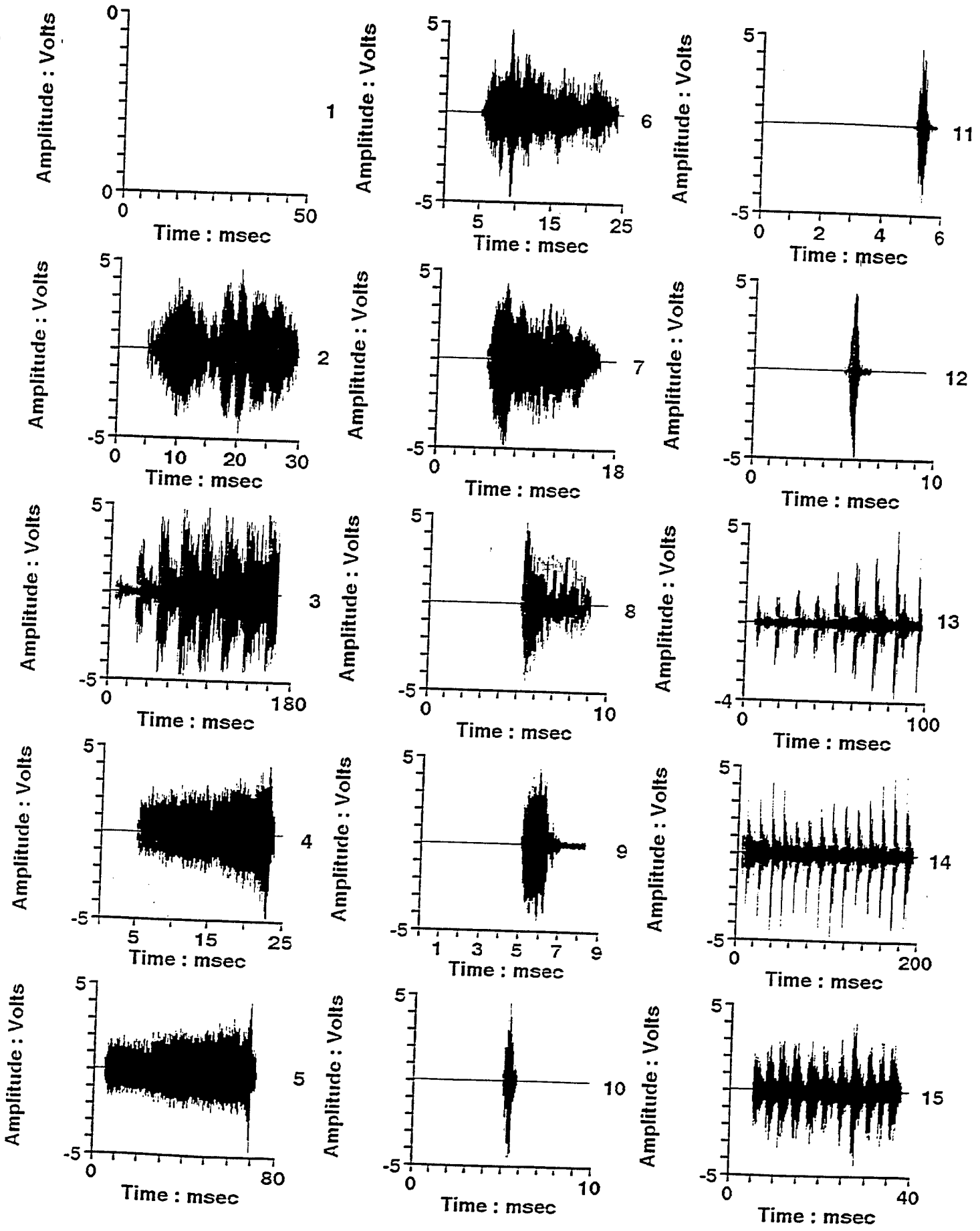


Figure 1. Amplitude oscillograms of communication calls emitted by the *Megaderma lyra*. Presentation of calls was preceded by a no-sound control buffer.

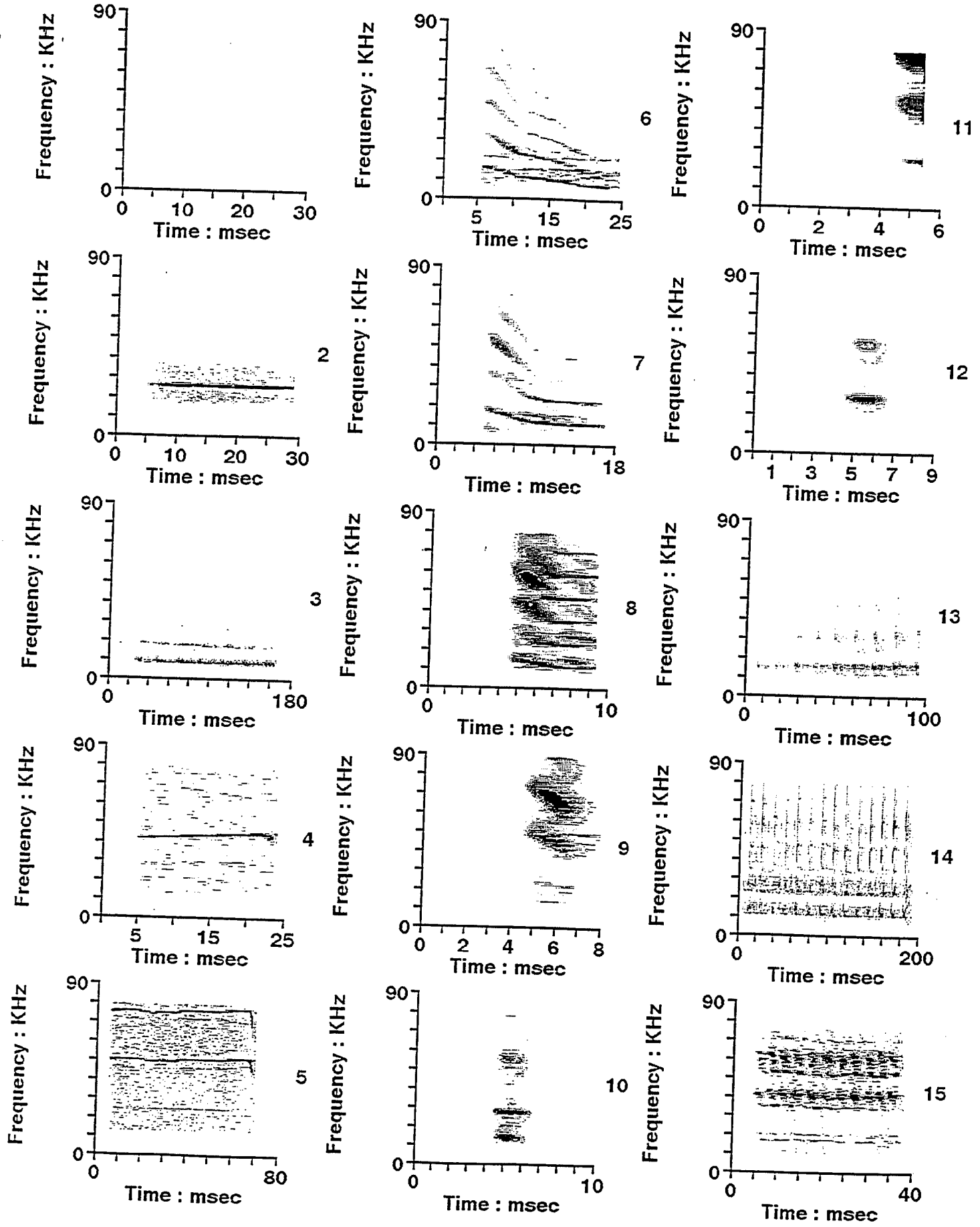


Figure 2. Spectrograms of communication calls emitted by the *Megaderma lyra*. Each call corresponds to an amplitude envelop shown in figure 1.

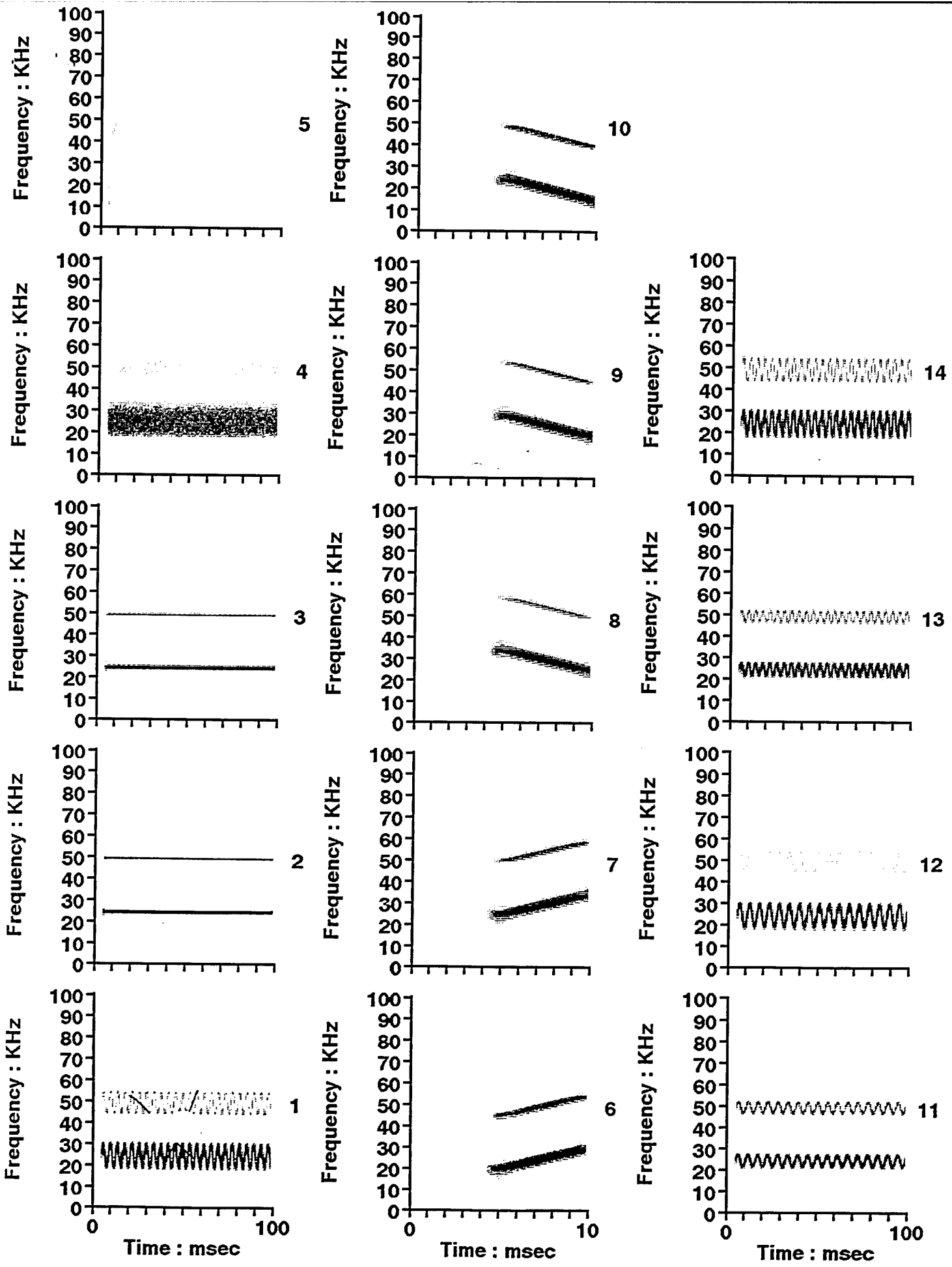


Figure 3: Basic acoustic patterns for presenting stimuli using SIGNAL routines.

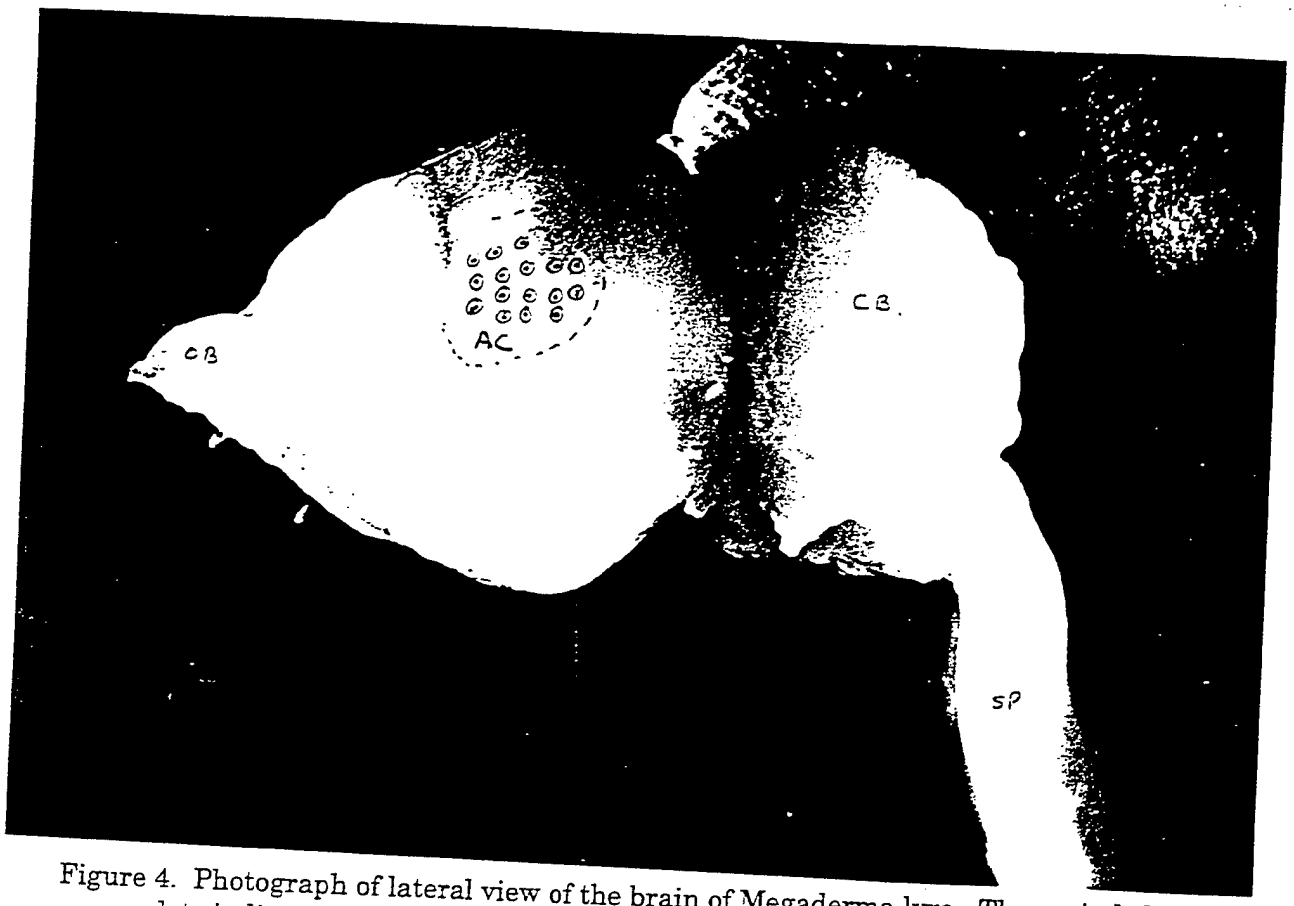


Figure 4. Photograph of lateral view of the brain of Megaderma lyra. The encircled dots indicate recording sites on the cortex from where auditory responses were obtained. AC: auditory cortex, CB: cerebellum, OB: olfactory bulb,. SP: spinal cord.

show both amplitude and frequency modulations. Figure 3 shows a set of 14 stimuli that were generated to study the basic response properties of these neurons. These sound stimuli represent the basic acoustic patterns that exist in nature and are adapted to be compatible with communication sounds of bats, both in the time and frequency domains.

Dr. Kanwal's research was the first attempt to record from the auditory cortex of this species (see figure 4). A preliminary analysis of the electrophysiological data from 16 cortical neurons suggest that some neurons in this species are multifunctional and respond to both communication and echolocation calls. Others are tuned only to respond to specific components in communication calls, e.g. slow rising frequency modulations. The most useful finding was that this species is well suited in terms of size and behavior for chronic recordings.

Dr. G.S. Chhina, Ph.D. in Human Physiology and Professor Emeritus at the "All India Institute of Medical Sciences" (AIIMS) joined Dr. Kanwal's group as a Research Associate to oversee the development of his new laboratory. This he has done and the laboratory is well on its-way to being functional despite construction delays. Over the long term, Dr. Chhina's expertise and experience will help to diversify the goals of Dr. Kanwal's laboratory to new research areas such as the representation and processing of music versus speech in the human cortex. Among other well publicized studies, Dr. Chhina has studied the role of music on cortical EEG in humans, Yogis, engaged in meditative practices. These studies will be extended on patients with brain injuries as well as normal humans using MRI technology as and when it becomes available at GICCS. To further facilitate studies in this area, Dr. Kanwal has invited Kyousuke Kamada, M.D., another Humboldt fellow, to join his group. Dr. Kamada was trained at the University of Hokkaido, Japan, and performed studies on rats and brain injury in humans. He is presently involved in NMR studies on communicative deficits in humans in Erlangen, Germany using a Siemmen's magnet, similar to the one that is planned to be acquired by GICCS. With this team of researchers, Dr. Kanwal will coordinate progress in our understanding of cortical processes for acoustic communication in both bats and humans.

JAMES J. PEKAR, PH.D.

Introduction

The Neurobiological Magnetic Resonance Laboratory, dedicated to the application of nuclear magnetic resonance to the study of brain anatomy, physiology, and function, was established during the last year. Accomplishments include construction, equipping, staffing, methodological implementation, and initiation of several research projects.

Construction of the 2000 square foot laboratory was completed, and the necessary occupancy permits acquired in early 1996. The seven Tesla superconducting magnet was delivered in March and energized in April.

Two postdoctoral fellows were recruited in the last year. Dr. Benedict C. Albensi (B.S. General Science, University of Oregon; M.A. Biology, Sonoma State University; Ph.D. Neuroscience, University of Utah) came in with a deep background in neuroimaging of neuroinjury and neuroprotection, as well as relevant experience in electrophysiology in hippocampal slice preparations. Dr. Benjamin G.M. Chew (B.S. Chemistry, California Institute of Technology; M.S. Physical Chemistry, Cornell University; Ph.D. Physical Chemistry, Cornell University) joined the lab with expertise in the theory and practice of nuclear magnetic resonance spectroscopy, and strong skills in related instrumentation.

The seven Tesla horizontal-bore animal MRI scanner is the highest-field unit of its kind in the greater Washington area and, thanks to hard work by the laboratory staff, is able to perform a variety of useful state-of-the-art methods (such as dual-coil studies, respiratory and cardiac gating, perfusion mapping, and isotropic diffusion mapping).

Several projects applying these techniques to questions in neurobiology are underway:

Project 1: Functional MRI studies of cortical plasticity.

Functional magnetic resonance imaging (MRI) non-invasively reveals brain areas involved in various tasks. We intend to use functional MRI to study cortical plasticity, by mapping how brain areas involved in sensory tasks change over time. Functional MRI allows repeated studies to be performed in the same animal over a period of time; these serial studies should give great insight into plasticity and also reduce the number of experimental animals needed, compared to other methods. Experimental elucidation of mechanisms of cortical plasticity is clinically relevant to recovery of neurological function following brain injury and to therapies for developmental disabilities.

The cortical representation of rat facial vibrissae demonstrates experience-dependent plasticity: clipping all vibrissae but one from birth ("univibrissa rearing") has been shown to enlarge the cortical representation of the remaining vibrissa.

This project is a collaboration with Drs. Josef Rauschecker, Jian-Young Wu, and Geoffrey Goodhill, all of the Institute.

Pilot data, demonstrating our ability to use magnetic resonance imaging to see changes in cortical NMR signal upon whisker stimulation, have recently been obtained (see figure 1).

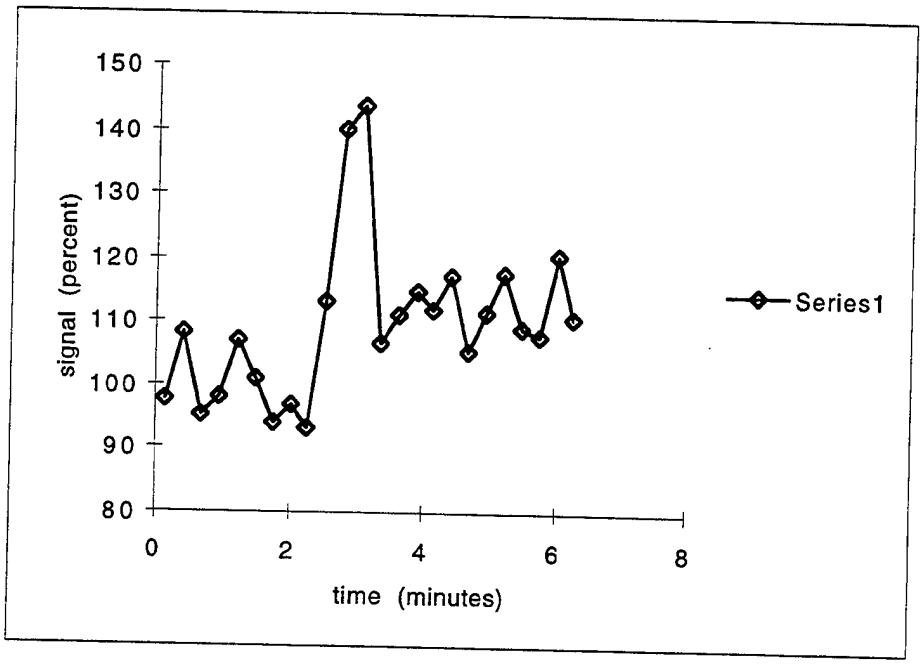


Figure 1: Nuclear magnetic resonance signal from region of rat primary somatosensory cortex before, during, and after stimulation of a rat whisker.

Project 2: Magnetic Resonance Microimaging of Cerebral angiogenesis in teratology

The teratological effects of thalidomide are hypothesized to be induced by early defects in angiogenesis. In collaboration with Dr. Gerald Goeringer of the Department of Cell Biology, Georgetown University Medical Center, we have initiated a project exploiting magnetic resonance microscopic examination of rabbit embryos exposed to this teratogen *in utero*, to examine defects in cerebral angiogenesis. Pilot data have recently been obtained in normal embryos (see figure 2).

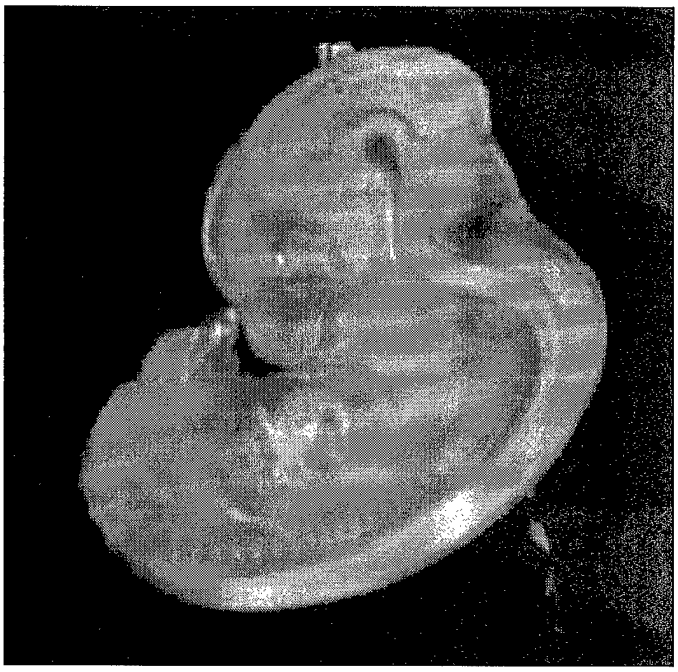


Figure 2: Magnetic resonance image of rabbit embryo. This image is a "virtual slicing" of an intact embryo, which was embedded in agarose gel and scanned for 40 hours.

Project 3: Magnetic resonance measures in traumatic brain injury

Use of modern magnetic resonance imaging techniques, especially diffusion and perfusion imaging, to establish and predict outcome in rodent models of traumatic brain injury, is being pursued in collaboration with Dr. Alan Faden of the Institute. We have recently obtained preliminary data which suggest that diffusion MRI may have utility for predicting secondary injury in these models (see figure 3).

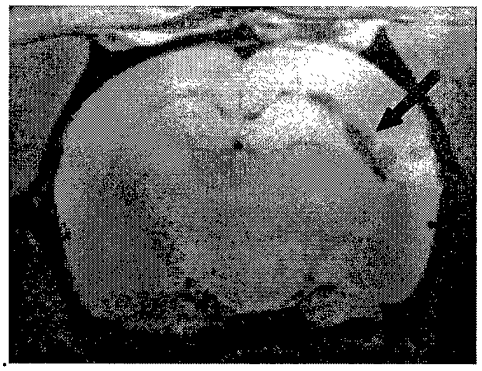
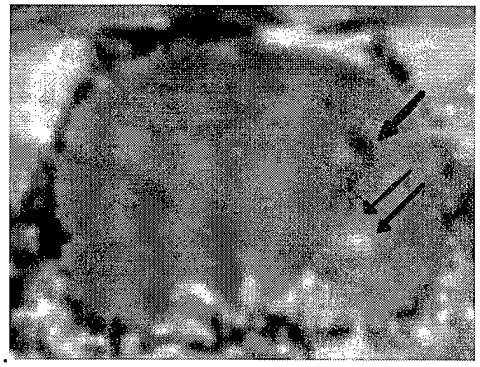
A.  B. 

Figure 3: Magnetic resonance images of rat brain, acquired in the coronal plane two days following fluid-percussion induced traumatic brain injury. A. High-resolution anatomical image (resolution = 78 um) showing region of hemorrhage (arrow). B. Diffusion map (pixel intensity represents value of the apparent diffusion coefficient for water) obtained from a set of diffusion-sensitized magnetic resonance images. Resolution is lower (312 um), but this map shows, in addition to hemorrhage (single arrow) a region of increased diffusion (hyperintense region; double arrows) which likely represents edema, and which is not resolved on the conventional image.

In addition to the projects already underway, several additional projects are planned:

Project 4: Functional MRI studies of auditory processing in the bat

In collaboration with Drs. Jag Kanwal of the Institute, we plan to use functional MRI to explore auditory processing in bats. Dr. Kanwal will maintain a colony of bats at the Institute for research purposes.

Project 5: Comparison of functional MRI and intrinsic optical imaging of the visual cortex

In collaboration with Drs. Jian-Young Wu and Josef Rauschecker of the Institute, as well as others, we plan to conduct parallel studies using intrinsic optical imaging and functional MR imaging of the visual cortex. The resultant data should shed light upon the nature and utility of the functional MRI signals.

Project 6: Magnetic resonance studies of early experimental allergic encephalomyelitis

In collaboration with Dr. John Richert of the Department of Neurology, Georgetown University Medical Center, we plan to use three-dimensional MRI to study the early course of experimental allergic encephalomyelitis in mice. This study should shed light on the utility of MRI for studying the early phase of this relapsing remitting demyelinating disorder and will pave the way for further studies of mice carrying human transgenes relevant to this (and other) brain disorders.

JOSEF P. RAUSCHECKER, PH.D., D.Sc.
BIAO TIAN, PH.D., AND C. MARK WESSINGER, PH.D.

Cortical Mechanisms and Plasticity of Auditory Processing

During the past year, efforts were devoted to completing the new laboratory structure for single unit electrophysiology and optical recording. Both facilities are housed in the same module on the podium level of The Research Building. Another recording suite with sound-attenuated chamber has been installed in the expansion project of the Research Resources Facility (RRF) and will begin to be utilized for chronic recording from awake behaving monkeys after the release of the animals from quarantine.

Dr. Tian has been in charge of setting up the optical imaging system (Imager 2001, Optical Imaging Inc.). It records the intrinsic optical signal change related to brain activity and reveals ensemble neuronal activity in the cortex at a large scale. This will give unparalleled insight into the functional organization of the auditory cortex. Several pilot experiments have been conducted. Currently, the system is undergoing minor modifications to enhance the imaging quality.

Dr. Rauschecker's group continues to explore the functional organization of multiple maps in the auditory cortex of higher mammals. The analysis of single unit responses to complex auditory stimuli in different cortical fields of cats and macaque monkeys has been expanded to include an evaluation of brain activation in comparable areas of human auditory cortex using functional magnetic resonance imaging (fMRI). As a third approach, further bridging the gap between single unit recording in animals and brain imaging in humans, optical recording is being performed in cats, with an option to extend this approach to humans in collaboration with the Department of Neurosurgery.

The lateral belt areas are part of auditory association cortex in the rhesus monkey. Neurons in these areas do not respond well to pure tones, but instead can be driven briskly by various types of complex sounds, including bandpass filtered noise (BPN) pulses and frequency-modulated (FM) sweeps (Rauschecker et al., Science 268, 1995). Both noise pulses and FM sweeps are essential components of many species-specific communication sounds, and neurons in the lateral belt areas often respond specifically to such sounds as well (Fig. 1). These areas may thus constitute a further stepping stone towards the analysis of auditory patterns, in particular those relevant for language evolution.

The use of chronic recording techniques also permits more complete mapping. The existence of three quasi-tonotopic maps along the lateral fissure was confirmed with BPN bursts (Fig. 2a). As in the anesthetized monkey, neurons in all three areas responded generally better to BPN and FM stimuli than to tone bursts ($p<0.05$; paired t-test). Most neurons were tuned to specific center frequencies as well as bandwidths. Best bandwidth (BBW) generally increased when the electrode was moved more laterally (Fig. 2b). Neurons in awake monkey also were specific for the rate and direction of FM sweeps. The degree of specificity and the overall preference of neurons in the three areas did not differ from that in anesthetized animals. Neurons in AL preferred FM rates around 50 Hz/ms, whereas ML preference was twice as high. Overall, 66% (46/70) of the neurons were FM-direction selective. When monkey vocalizations were used for stimulation, temporal and/or spectral combination-sensitivity was found in 7 out of 34 neurons. In 15 out of 32 neurons the response to the original vocalization was at least twice as good as the same vocalization played backwards, which indicates a fair amount of selectivity for monkey calls.

Over the past year, Dr. Rauschecker's laboratory has concentrated on exploring the neural mechanisms that generate response selectivity for such species-specific vocalizations. It was found that non-linear summation in the frequency and/or time domain is one very important mechanism: neurons often do not respond to single components of a monkey call, but are driven vigorously when two or more components are played together (Fig. 3) or in succession (Fig. 4). Reversing the temporal order also abolishes the response in many instances. Similar response properties have been described in the auditory systems of songbirds, bats and frogs and have been termed "combination-sensitive." More specifically, using monkey calls (MC), we found 40% (79/196) of the neurons in the lateral belt areas were selective (MC preference index ≤ 3) to monkey vocalizations (Fig. 5). Of the neurons we tested, 8 out of 42 neurons (19%) were spectrally combination sensitive, 5 out of 63 neurons (8%) temporally combination sensitive, and 17 out of 61 neurons (28%) were reversal sensitive (Fig. 6). It is the first time that such neurons have been found in primates, and it can be inferred that the decoding of speech sounds in humans is based on similar neural mechanisms (Rauschecker, 1996).

Consequently, we are using similar types of stimuli in our fMRI studies of human auditory cortex. Human speech sounds ("phonemes") are composed of information-bearing elements, such as BPN pulses and FM glides, which are in many ways similar to the subcomponents of monkey calls (Fig. 7). The initial results, acquired by Dr. Wessinger on the 1.5T Siemens Vision scanner at Georgetown University, indicate distinct and localized activation by BPN and FM stimuli, whereby the area activated by BPN comprises a region larger than that activated by pure tones.

In addition to exploring the functional specialization of different maps in the auditory cortex, Dr. Rauschecker's group is also testing the hypothesis that the central auditory system is organized in similar dual fashion into a spatial and a pattern system, as found in the visual and somatosensory systems. In monkeys, his group is combining the electrophysiological approach with the use of neuroanatomical tracers in order to characterize the input-output connections of the cortical maps and relate them to their physiological response properties (Rauschecker et al., 1996).

In humans, the dual pathway hypothesis is again put to the test by means of functional brain imaging. In studies using positron emission tomography (PET), sound localization of "virtual auditory space" stimuli (generated by computer and incorporating spectral information from head-related transfer functions in addition to binaural information) consistently activates a center in the right inferior parietal lobe (IPL). At the same time, the rostral portion of the superior temporal gyrus (STG) gets deactivated, indicating involvement in auditory pattern processing (Weeks, et al., 1997). The plasticity of this system is underscored by the fact that the size of this area of activation in the IP is significantly enlarged in congenitally blind people and extends far into normally visual areas (18 and 19) in the occipital lobe (Aziz-Sultan et al., 1997). These recent PET data from blind humans tie in extremely well with prior results from Dr. Rauschecker's lab on visually deprived cats (Rauschecker and Korte, 1993; Rauschecker, 1995).

In conclusion, the Cognitive Neuroscience approach, in which parallel animal and human studies are planned and conducted together, tackling the same question about higher cognitive processing at different levels of analysis, has already proven highly successful at Georgetown. Further examples of this approach will become evident as collaborations emerge between faculty members using neurophysiological testing in animal models and those using functional imaging in humans.

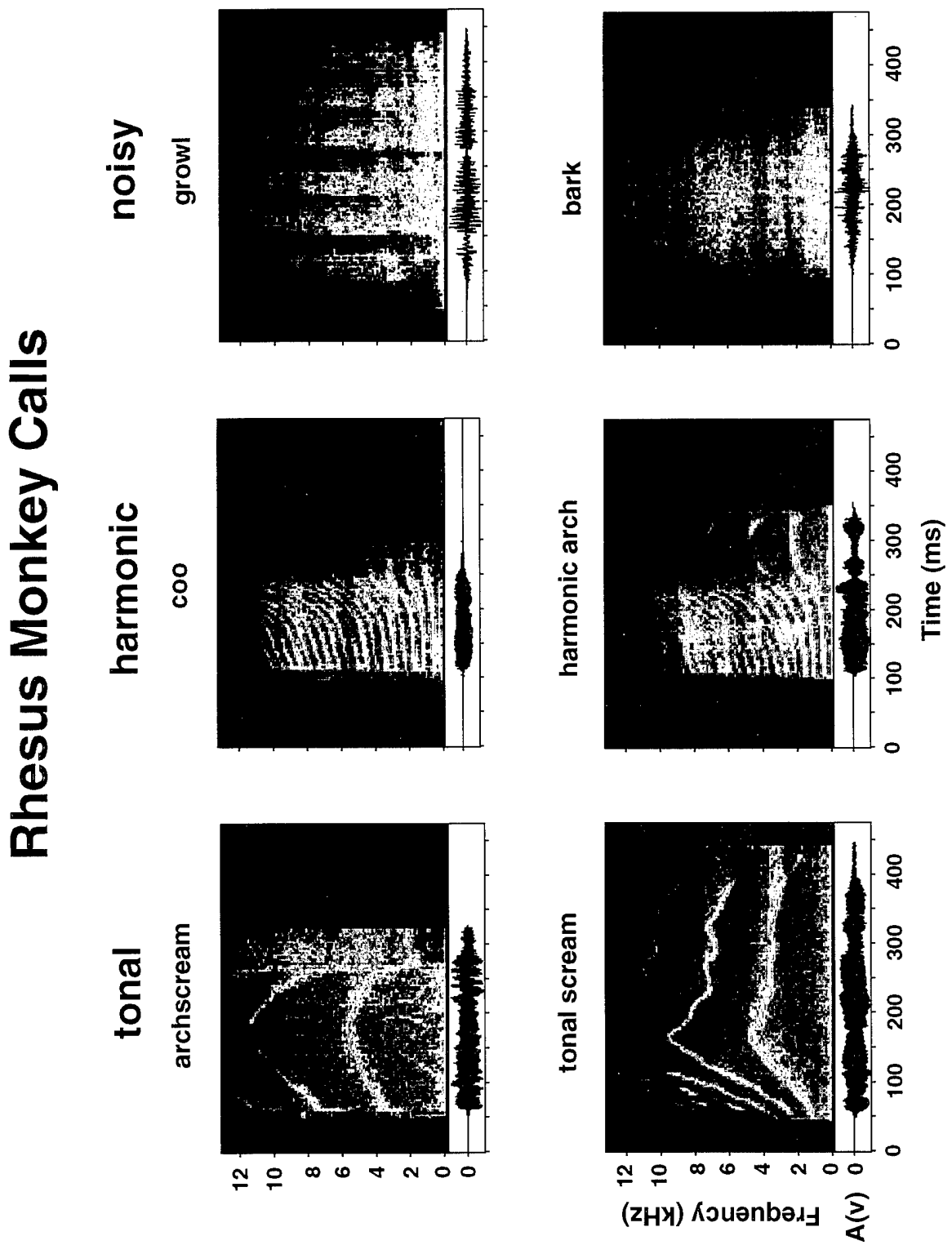


Figure 1: Representative examples of rhesus monkey vocalizations. Spectrograms display frequency content over time. The corresponding amplitude signal is shown underneath each spectrogram. Monkey calls fall into three major categories: tonal, harmonic and noisy.

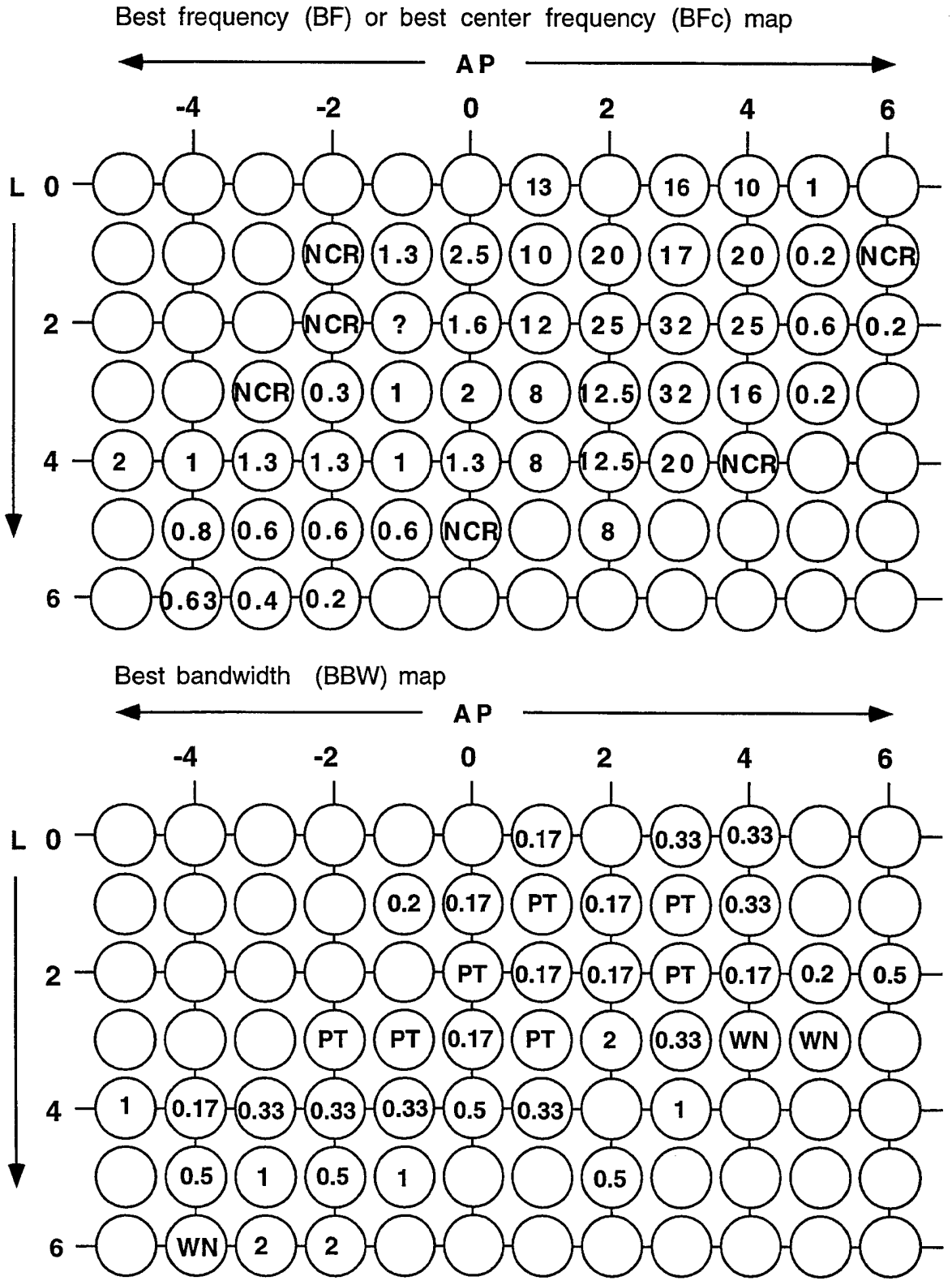


Fig. 2

Figure 2: Maps of best center frequency (top) and best bandwidth (bottom) recorded from the supratemporal plane and superior temporal gyrus in a monkey implanted with a chronic recording chamber.

Spectral Combination-Sensitivity

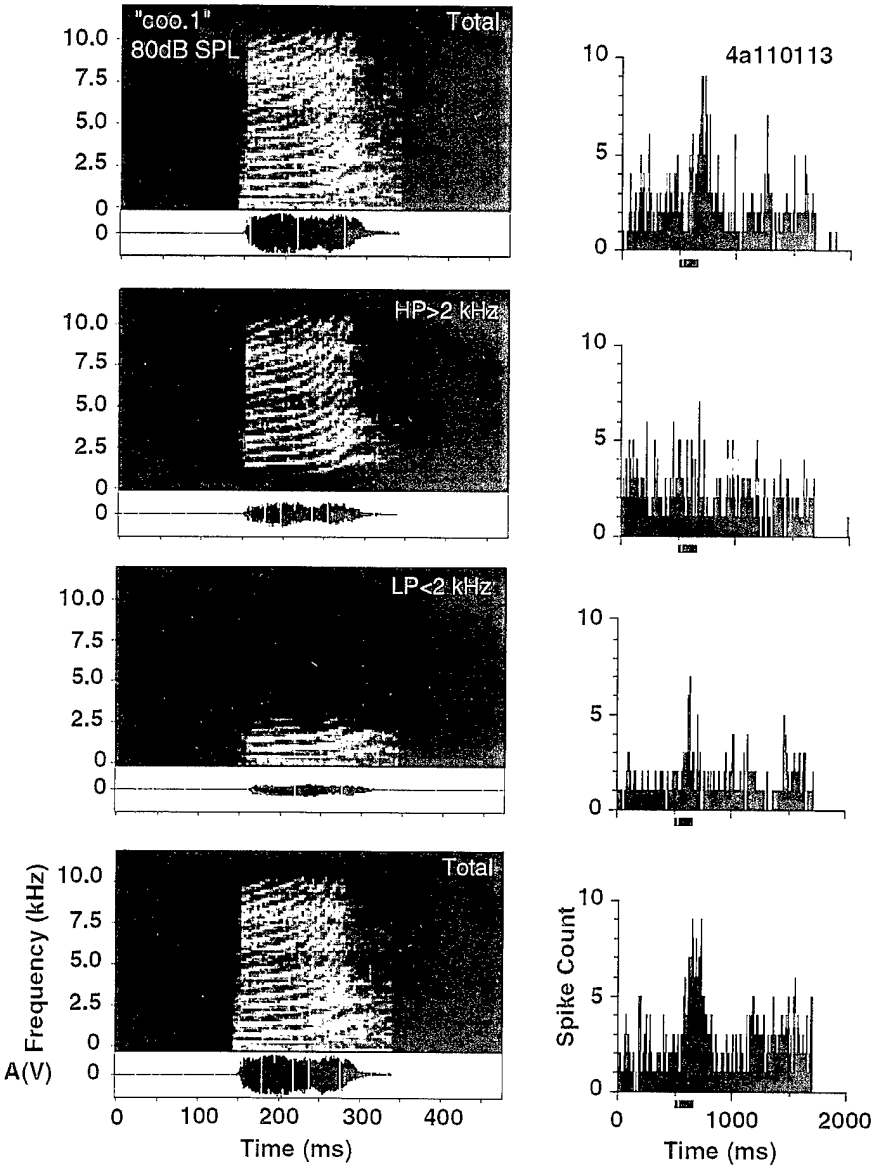


Figure 3: Spectral combination sensitivity in a neuron of the lateral belt areas. The spectrograms of the stimuli are shown in the left column, the corresponding neural responses (in the form of peri-stimulus time histograms) are shown on the right. A "coo" call (top and bottom) evokes a good response in the neuron. High-pass and a low-pass filtered versions of the call do not evoke the same response.

Temporal Combination-Sensitivity

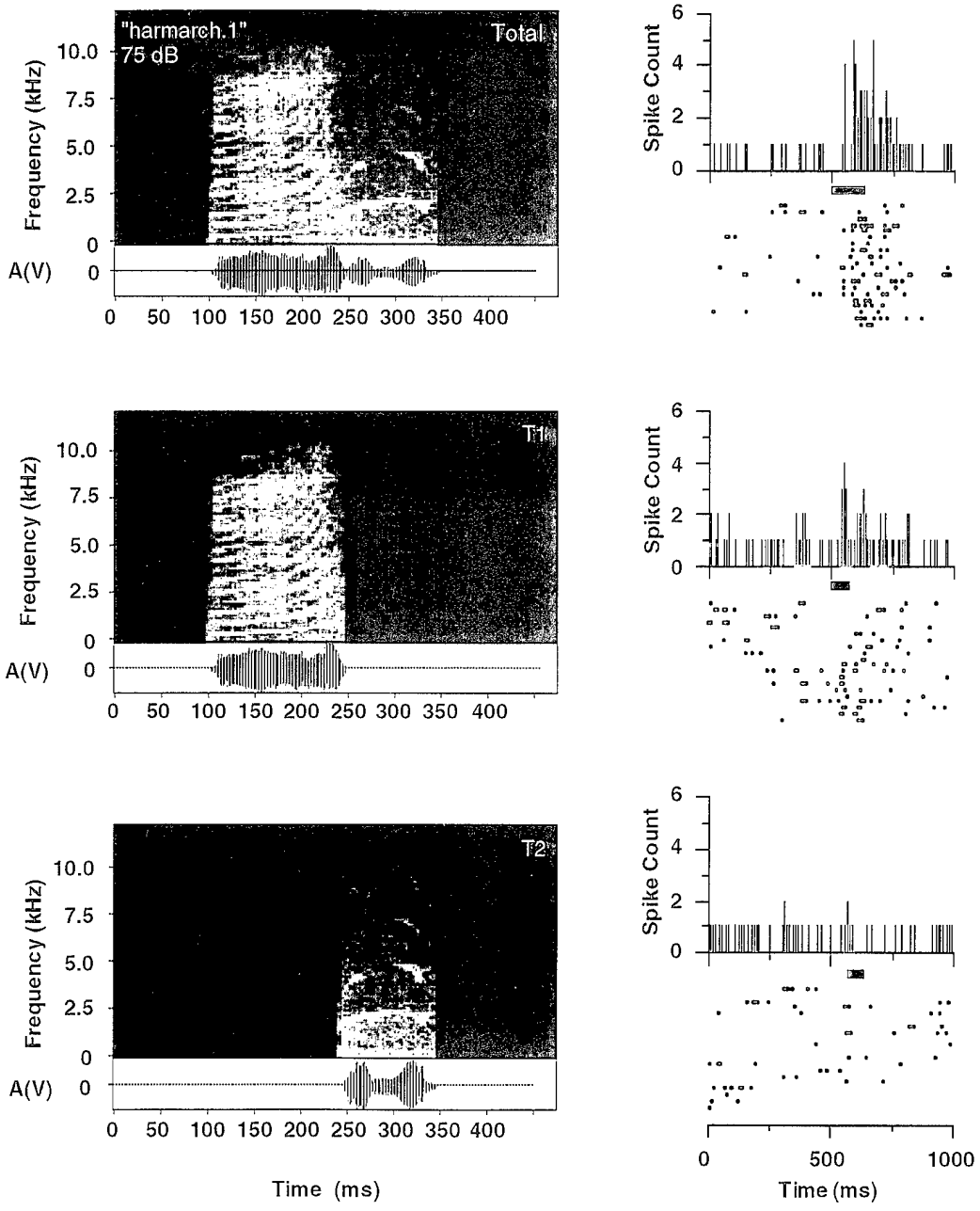


Figure 4: Temporal combination sensitivity in a neuron of the lateral belt areas. Again, the spectrograms of the stimuli are shown on the left, the response of the neuron (PSTH and raster display) on the right. Species-specific vocalizations or portions thereof were used. At the top, the response of the neuron to the whole stimulus (a "harmonic arch") is shown. The bottom two examples show the response to the two elements ("syllables") when played back separately.

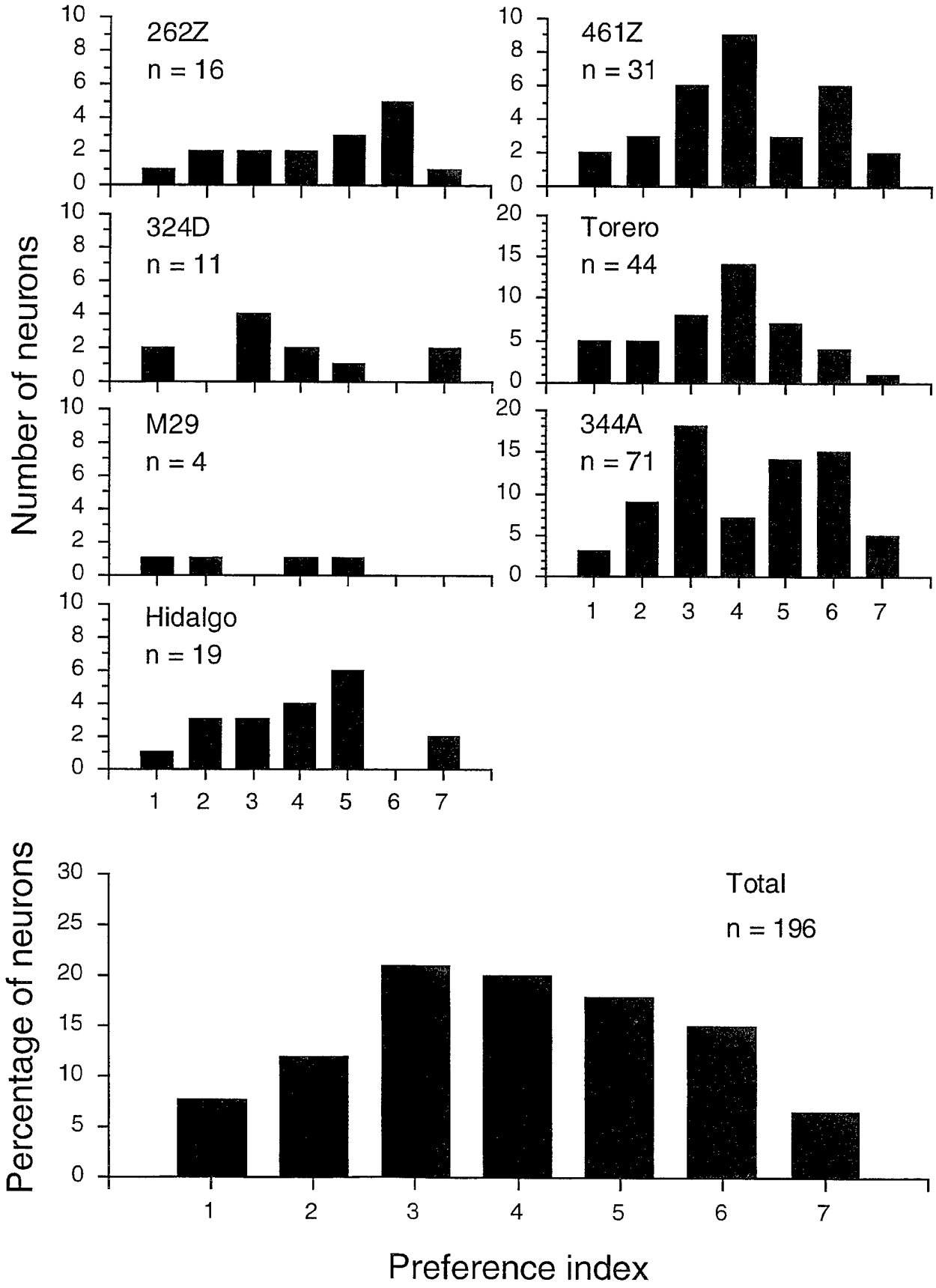


Figure 5: Summary diagrams from seven different monkeys displaying the preference of neurons in the lateral belt for monkey calls. Few neurons respond only to a single call. Most respond equally well to 3 or 4 calls. This could indicate that communication calls are coded by small networks of neurons rather than single "grandmother" neurons.

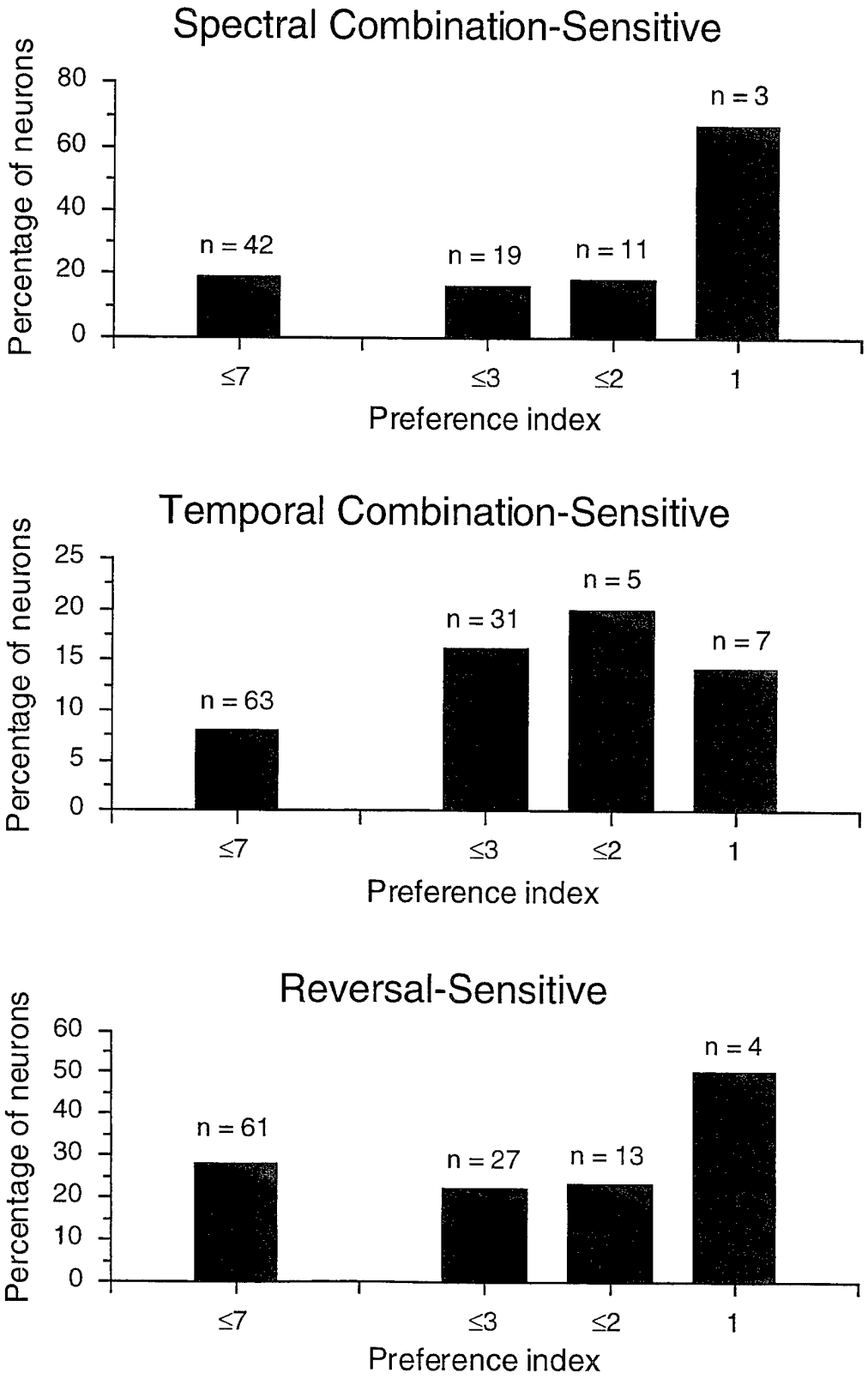


Figure 6: Analysis of the mechanisms leading to response selectivity indicates nonlinear summation. Proportion of neurons displaying combination sensitivity (Figs. 3 and 4) or reversal sensitivity. The proportion of such neurons is higher among neurons with greater selectivity.

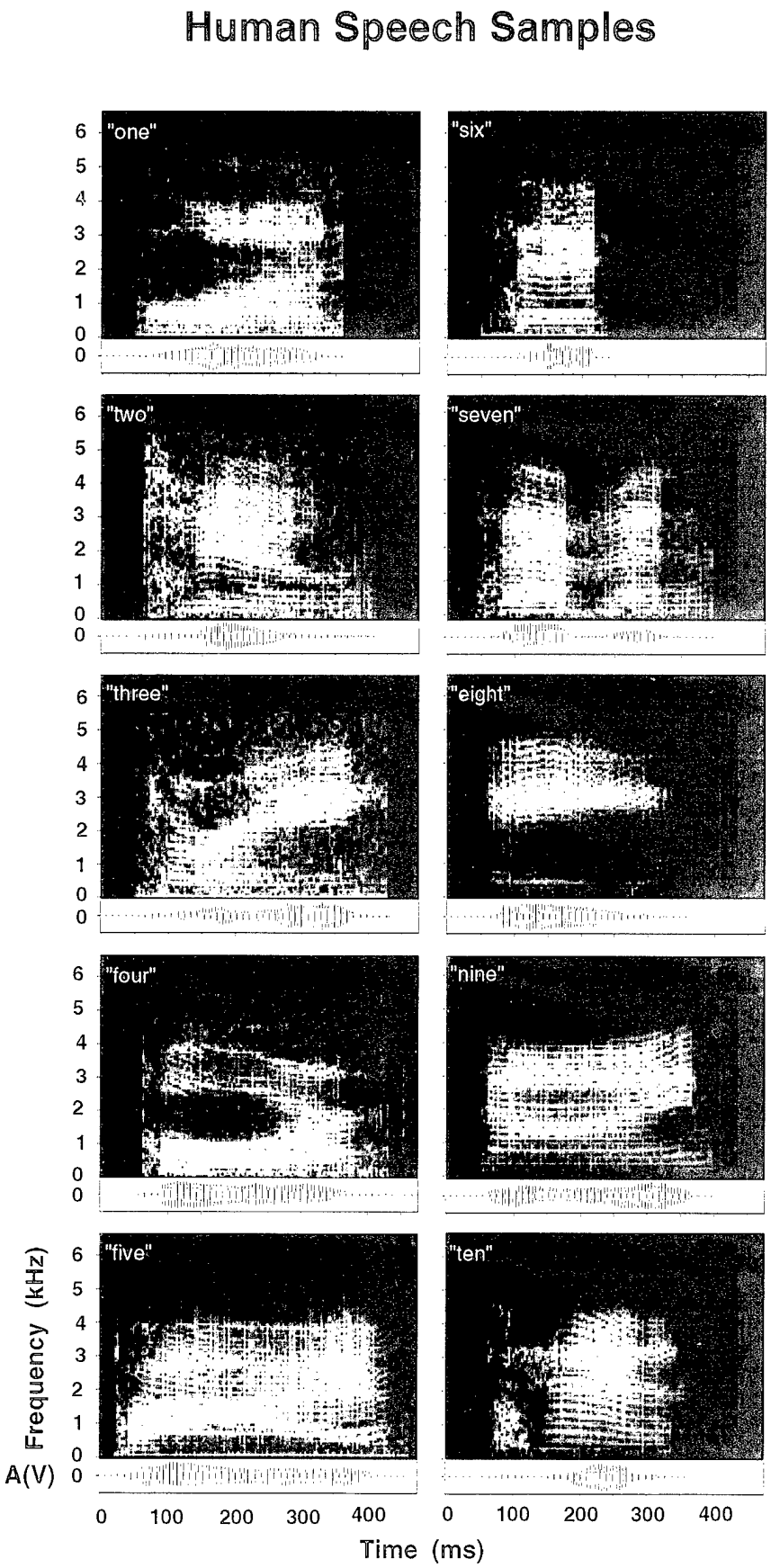


Figure 7: Human speech samples show some of the same characteristics displayed by animal vocalizations. Studies in animals therefore serve a useful role in helping to understand the neural basis of speech perception.

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Abstracts

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Weeks, R.A., Tian, B., Wessinger, C.M., Cohen, L.G., Hallett, M. and Rauschecker, J.P. Identification of the inferior parietal lobule as the site of auditory space perception in humans. American Academy of Neurology (1997) (in press).

JIAN-YOUNG WU, PH.D.

The GICCS Optical Imaging Laboratory experienced great progress during the past year. The equipment, including three kinds of optical imaging devices, has been completed, and three staff members have been recruited. With our major interest in dynamic neural organization and synchronized cerebral events, two ongoing projects and two collaborating projects are generating data.

1. Laboratory Development

Equipment

Three sets of apparatus for optical recordings have been completed during the last budget period:

-- A 124 Channel diode array system is fully functioning and has been our major set-up for fast voltage sensitive dye imaging.

-- A cooled CCD camera (Princeton Instrument) system has been completed. The system can run as fast as 180 frames per second so that the fast transient optical signals can be recorded and digitized. The system has been equipped with conventional camera lenses that allow us to image at low magnification and high numerical aperture at low cost. We used the system for monitoring and taking optical data from brain slices and believe this is a highly potential device for optical imaging in the future.

-- A Dage CCD camera and an image processor have been installed and are being used for processing intrinsic optical signals. This is a successful innovation for using an ordinary image processor to process intrinsic optical signals on line. With this device for the first time we are able to use ordinary video tape to record intrinsic optic signals that before would have taken giga-bytes of computer disk space.

Computers and Internet connections

All the computers in our lab, including office computers and specialized experimental control computers, have been configured and connected to the Internet. A UNIX (Linux) workstation (named fractal.giccsw.georgetown.edu) has been installed. It functions as our personal server for e-mail, an ftp site, NFS, PPP, SLIP, and WWW and to exchange information with scientists throughout the world. Some

GICCS faculty and staff members are also regular users of this machine. MATHEMATICA, a comprehensive mathematical package, is installed on this machine and will assist all the users with data processing and modeling.

Machine shop / Electronic work bench

We have built a small shop in our laboratory with a mill machine, a drill press and basic electronic testing instruments. This shop is very helpful for building our new laboratory. We have done a lot of work such as fabricating recording chambers, modifying optical bench parts and making simple electronic devices. People from other laboratories often come to work in our shop.

Recruiting and laboratory personnel

Three people have been recruited since last year.

Dr. Yang Tsau (Yale University) joined the laboratory last March as a research associate scientist. Dr. Tsau has contributed his expertise with computer systems and networking to our ongoing projects.

Dr. Li Guan (University of Maryland at College Park) joined the group last September as a postdoctoral fellow. Recently she found the non-linear migration and attractions between temporary active columns; we were extremely excited about these findings.

Ariane Schaefer joined the group last July as a research assistant. She has extensive laboratory experience in physiological research and animal models.

We have formed an integrated group using novel optical recording techniques to understand basic cortical processing during normal cognitive functions and abnormal situations in organized epilepsy.

The major goal of cognition science is to understand the internal representation of mental events. At the cellular level, each individual cerebral neuron can represent only a small portion of an event. Thus, examining the collective activity of millions of neurons as a whole becomes a fundamental strategy for system neurobiologists. We analyze the coherent and large scale neural events using optical imaging techniques rather than recording from individual neurons (a metaphor would be viewing a whole TV screen versus analyzing the intensity of individual pixels). Voltage sensitive dye imaging, as our major approach, directly reports the electrical activity of the neurons and can have a temporal resolution of 0.5 ms to follow fast neural events. The following projects are aimed at understanding the dynamic neural assemblies in cerebral cortex slices.

Project 1: Dynamic cortical neural organization

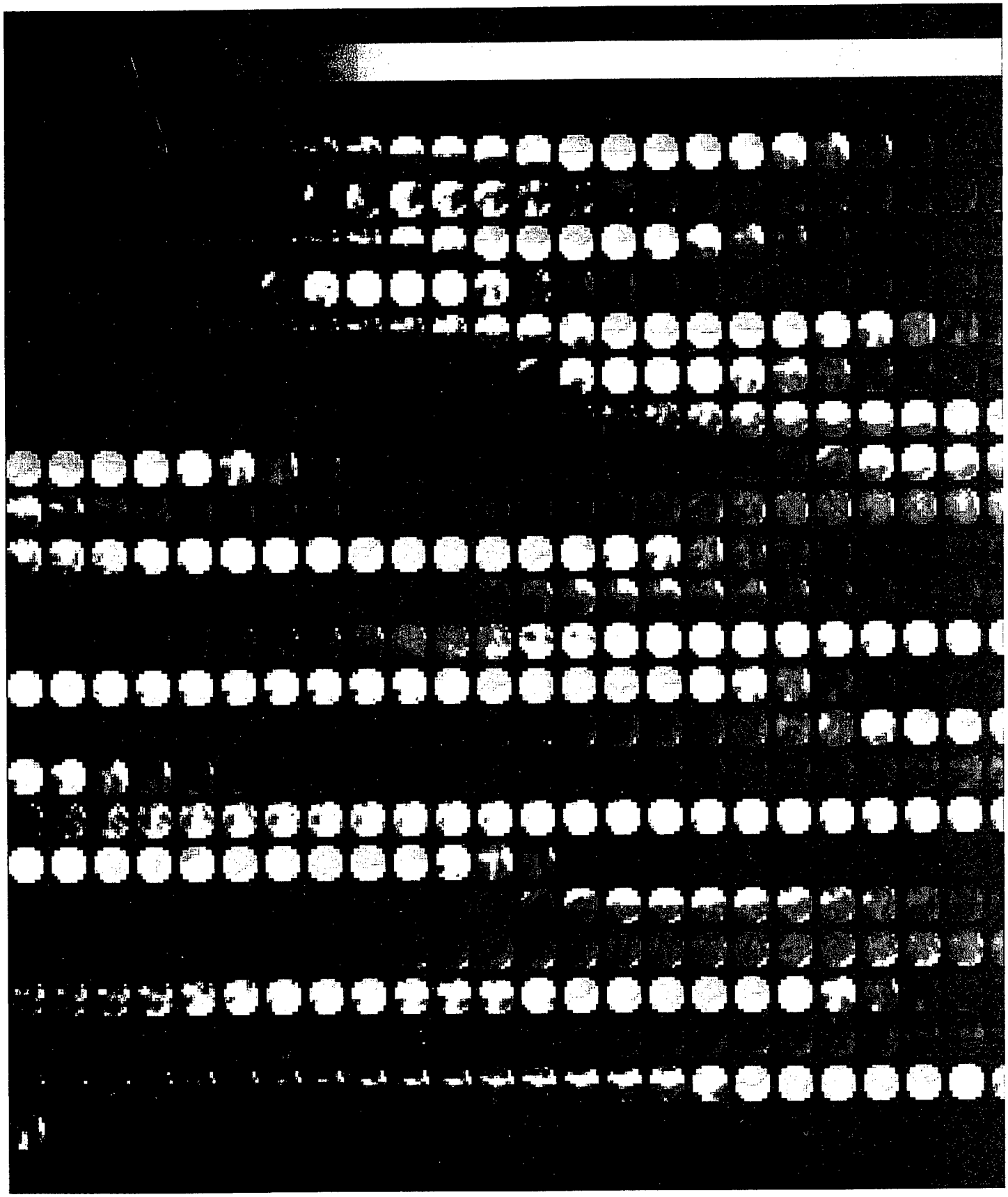
Introduction

The long-term objective of this study is to understand localized cortical interactions for information processing and storage. Coherent and oscillatory activities may play important roles ranging from complex pattern perception to organized epilepsy. During oscillations, temporary cortical neural organizations have never been visualized. My speculation would be that temporary cortical assemblies may be different from afferent driven cortical columnar organizations. The proposed organizations for the temporal neural assemblies may be either distributed or localized. In the distributed organization neurons which follow the oscillations are distributed among the non-follower populations, as suggested by correlation analysis of microelectrode recordings. In the localized organization large portion of neurons are coherently active during certain tasks, as suggested by recent intrinsic optical images for the cortex . We use fast imaging (0.5 ms per frame) with voltage sensitive dyes and electrode array to study the spatio-temporal dynamics in rat cortical slices. During the past year we have found that the spontaneous epoch oscillations in cortical slices are localized temporal assemblies with self-sustained coherent activity.

The neocortical slice is a new preparation for our laboratory. In order to obtain stable experimental conditions and reliable data, substantial efforts were made in order to optimize our apparatus and experimental method. After seven months of effort, we have accumulated a great deal of knowledge and skills. These include preparing the slices, keeping them healthy for a long time, staining with voltage sensitive dyes, optical recordings and electrode array. Now almost every slice we prepare is healthy, well stained and yields large optical signals.

TEMPORARY ACTIVE COLUMNS, HIGH MAGNIFICATION 1

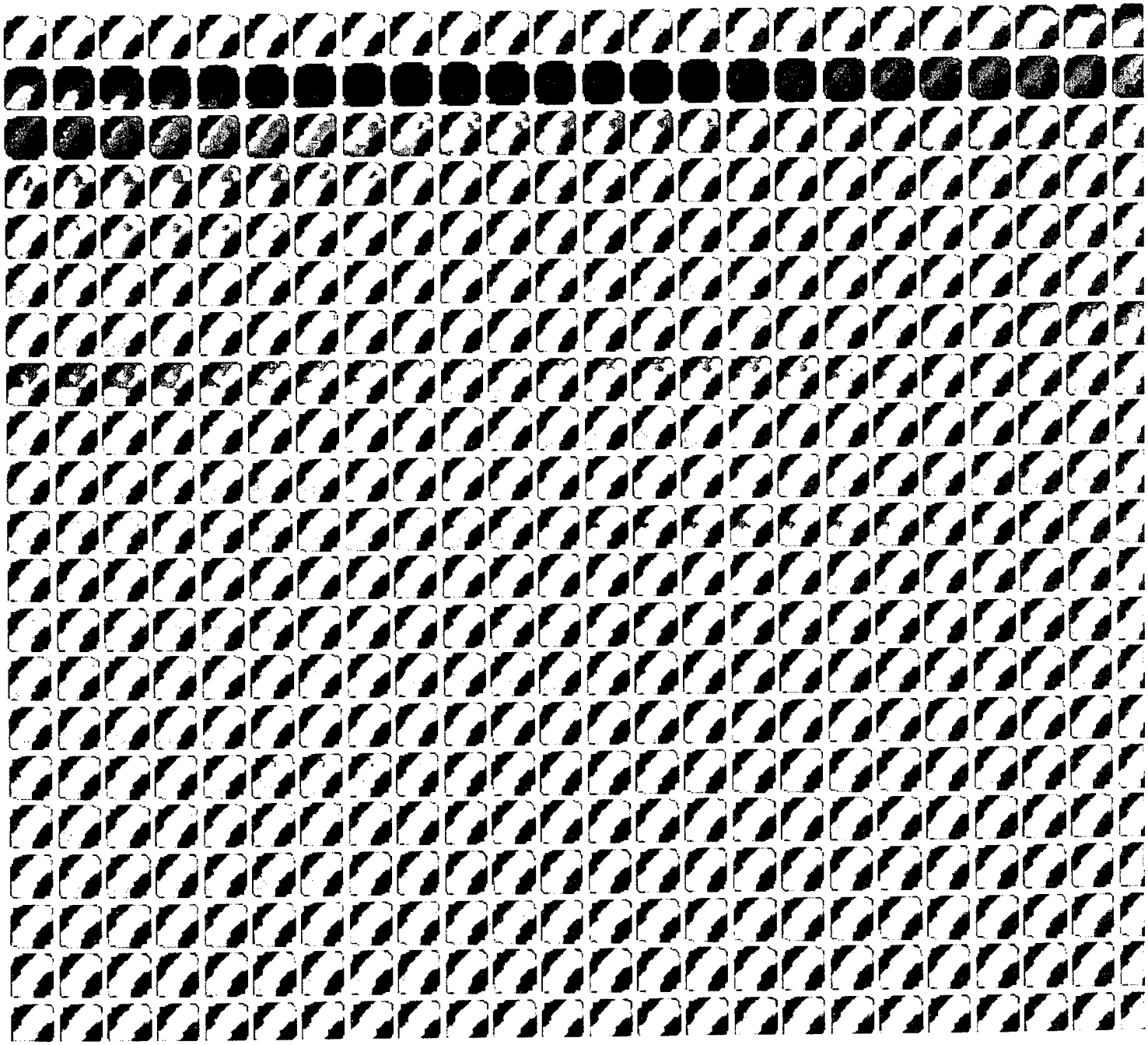
6, 400 MS SEQUENTIAL MULTI-FRAME DISPLAY, 12 ms per frame



Pixels Max=213min=-272 fixed scale smoth= 1 Start= 9586 step = 12 ms 576 frames Y= - 38

TEMPORARY ACTIVE COLUMNS, OVERVIEW 2

5,000 MS SEQUENTIAL MULTI-FRAME DISPLAY, 10 ms per frame



r6080601.log frame start=10469 step=10ms total=5040ms

wumodles.pre

Experiment

Optical images of the preparation at 705 nm were obtained by a 124 element diode array at a sampling rate of 1 ms per frame. For spontaneous events we took 16 seconds of optical recording trials. Each trial contains 16,000 sixteen-bit images. The data were stored in a computer and analyzed by a program developed from IDL high end programming language.

We have examined the spontaneous epoch oscillations in neocortical slices in low magnesium media. This event was first described by Connors and co-workers, who thought that oscillations were initiated only by local interactions in cortical V (Silva et al , 1991). Using optical imaging, we found that epoch oscillations were actually composed of two parts. The initial part was a global propagation wave spreading over the entire preparation. Oscillation loci emerged a few seconds after the initial wave. The oscillations did not spread, but formed centers of active areas. We were surprised that we were able to obtain such beautiful optical recordings from spontaneous oscillatory events (Figure 1, high magnification and Figure 2, overview). The signal-to-noise ratio was so high that oscillations were easily distinguishable in single trials without averaging or other signal processing. This high signal-to-noise ratio made it possible for more sophisticated experiments to further explore the dynamic structure of oscillation loci and to examine whether these loci were connected to localized neural assemblies or to attractors as speculated by theorists.

Results : Multiple loci of oscillations

Voltage imaging reveals that the oscillations are localized in one region. In each epoch of oscillations, several loci may co-exist at different locations. In Figure 1 we can see at least two possible oscillation centers. One has its center in the field of view. Another center is outside the field but its activity can still be seen. Multi-electrode recordings support this observation. With two electrodes, we have sometimes recorded oscillations from different loci. When two electrodes were separated by about 2.5 mm, the oscillation patterns recorded were very similar. However, when the record is examined in the expanded time scale, the oscillations from the two sites appear different.

The observation that more than one locus can coexist in one slice raised several questions about the localized oscillations. I. How many loci are in one cortical section? II. Do oscillations always emerge from fixed locations? If the loci are fixed, are they related to cortical columns and other cellular architecture?

In a new set of experiments, we had a larger field of view so that a large portion of the cortical section could be examined. In this configuration more features of the oscillation have been revealed. We found that during the oscillations, more than one cluster of bursting neurons formed at different locations of the hemisphere section. These loci have an approximately columnar structure and we call them "localized active columns (TACs)". TACs emerged (Figure 2) at the beginning of each oscillation cycle, were sustained for a about a half cycle (50 to 100 ms) and then vanished during the other half cycle. At the beginning of the next cycle, clusters with different sizes could reappear from different locations (Figure 2). This dynamic process is accurately in phase with each oscillation cycle.

A typical TAC has a columnar structure with base width of 0.5 to 4 mm. Vertically, it incorporates all cortical layers. During its lifetime the base width may change significantly.

The neural activity (either the number of active neurons or the average bursting rate per neuron) was higher in the center of the TAC column. The column had a clear boundary at which the number of active neurons or the average firing rate dropped sharply (Figure 1). It is not distinguishable with our images if there is an inhibitory band surround the active column.

Most of the TACs drifted away from their emerging place. Some moved as far as 10 mm across the boundaries between anatomically distinct cortical areas. The moving direction and range seemed irregular; even during different oscillation cycles of one epoch event, they could move in different ranges and opposite directions.

Often more than one TAC loci emerged from different locations in phase with one oscillation cycle, and attractions between them could be seen. They moved toward each other and merged into a new shape (Figure 2, e.g., the events in the second row from the bottom). Attraction made the TAC traveled much faster (some 5 to 10 times faster) than the random drifting which occurred when no attraction was present.

A very interesting question is whether oscillation loci are related to the activity history of the cortical circuitry. In many cases we see TACs emerge from the same location during different oscillation cycles. However we are not sure whether these locations had been modified by previous activity history or whether they overlapped with cortical columns during afferent driven events. We will use micro-stimulation on the

slice to induce a new locus or alter existing loci. Ultimately (beyond the scope of this project) we will examine if natural sensory stimulation elicits oscillation loci in the cortex.

Results: Dynamic structure of oscillation locus

What is the firing pattern of neurons within a TAC? We have proposed two possible temporal patterns: One is that a fraction of neurons in a locus may be active synchronously on a millisecond scale (temporal coding hypothesis). Another is that neurons in an assembly are not firing coherently on a millisecond scale but coherently raising their firing rate together (Attractor hypothesis). Voltage imaging can be used to distinguish these two forms of coherency. With our optical magnification (10 X objective) each photodiode can detect the soma and dendritic membrane of thousands of neurons. If a fraction of neurons in the field of view are firing together in a few milliseconds, a large spike several milliseconds wide would appear in optical signals. However, if there is a non-synchronized rise in the spiking rate of a large number of neurons, we will see a slow rising population signal. In our current data we only see slow rising signals but never large, sharp spikes (Figure 1) suggesting that TACs do not have a highly synchronized dynamic structure. In our proposed experiments, we will directly measure the firing rate of the individual neurons in the center of the locus to further verify the temporal pattern of spiking rate change and synchronization.

The spatial distribution of activity is also suggested by voltage images of oscillation centers, as shown in Figure 1. There is a sharp decrease in activity from the center of a TAC to its surrounding area. This decrease may mean the average firing rate decreases from the center to the surrounding area and/ or the number of active neurons decreases from the center to the surrounding areas. To verify this observation, we plan to use several microelectrodes to simultaneously measure the firing rate of individual neurons in the center of the locus.

Voltage imaging does not detect current flow between source and sink. Thus the size of the active area imaged is accurately correlated with the actual active area. Our proposed experiments will also use high magnification voltage sensitive dye imaging to examine the detailed spatio-temporal pattern of an oscillation center.

Conclusions

The existence of dynamic neural assemblies, as proposed since Hebb, remain largely speculative. Voltage sensitive dye imaging showed that during spontaneous epoch oscillations in neocortical slices bathed in zero magnesium medium, cortical neurons formed temporary active columns (TAC). The TACs varied in size (0.5 to 4 mm wide) and incorporated all cortical layers, with higher activities in the center. In phase with each oscillation cycle, multiple TACs emerged in different regions of the coronal section of the hemisphere. The activity was sustained for only about one half of an oscillation cycle (50 to 150 ms). New TACs different in location and size emerged during the next cycle. Some TACs stayed where they emerged and some drifted across a long range. Two TACs were often attracted to each other, first moving towards each other rapidly and then merging. TACs are temporal neural assemblies, and since the activity column varies in size and may move across a long range, it may be organized by an activity dependent non-linear excitation-inhibition balance. This form of cortical assembly seems to be different from the cortical columnar organizations described during afferent driven events.

Part of this data has been submitted as the preliminary results in an application for a NIH R-01 grant. We are currently writing a paper for the publication of these results.

Project 2: Initiation and spreading of seizure-like activity in cortical slice preparation

Introduction

The initiation of synchronized oscillatory events is an important issue in understanding the development of organized epileptiform discharge. Presently the way in which synchronized events arise from local neural activity remains largely speculative. Although abnormal excitability in the neural network is important for the synchronized discharge, a special process is needed for recruiting localized activities into a paroxysmal epileptiform discharge capable of being self-sustaining and propagating. In theory a brain slice bathed in zero magnesium solution should have the hyper-excitability homogeneously distributed over the whole neural network with millions of neurons. Epileptiform epochs could start from anywhere or

everywhere in the preparation. So why do synchronized epoch events always started from a few dominant centers? How do these centers become dominant and do they suppress other places from becoming centers? These intriguing questions have not received adequate experimental attention due to the lack of fast imaging methods to visualize the initiation of epileptiform events and to analyze the organization of neuronal activity which take place before synchronized events occur.

There are a number of ways in which synchronized events could arise from local activity. The "pacemaker" hypothesis assumes that with certain excess activity, local neural connections are potentiated to form a pacemaker circuit which starts synchronized events. The "resonance" hypothesis suggests that a special local activity pattern may cause extensive resonance of a large population of neurons; a synchronized event is the result of such resonance. The "interaction" hypothesis proposes that the interaction of small scale discharges causes an all-or-none event. There is also the possibility that changes in the local extra-cellular space (ECS), due to secondary effects of excess activity, may directly recruit neighboring neurons to form an initiation site of synchronized events. During the past year our experiments have partially verified some of these hypotheses.

Identifying ways to disturb the initiation of epileptiform organization may have substantial clinical significance. Our experiments will test the "shock-to-stop" idea in our slice model system by attempting to answer three questions: I. Can one induce many small and local events to reduce the chance of a large and global event ? This includes the possibility of introducing break-up ictal discharges into many inter-ictal spikes, thus preventing organized seizure events by inducing many localized discharges. II. Does a seizure initiation site have a special "hardware" connection among neurons? If it does, can one use micro-shocks to modify the network connections and to eventually destroy the circuit for initiating seizures ? III. Are the location and timing for introducing the stimulus important in stopping a seizure event? These questions will be studied in the brain slice preparation in this proposal and eventually (in subsequent projects) in experimental epilepsy models in vivo.

Experiment

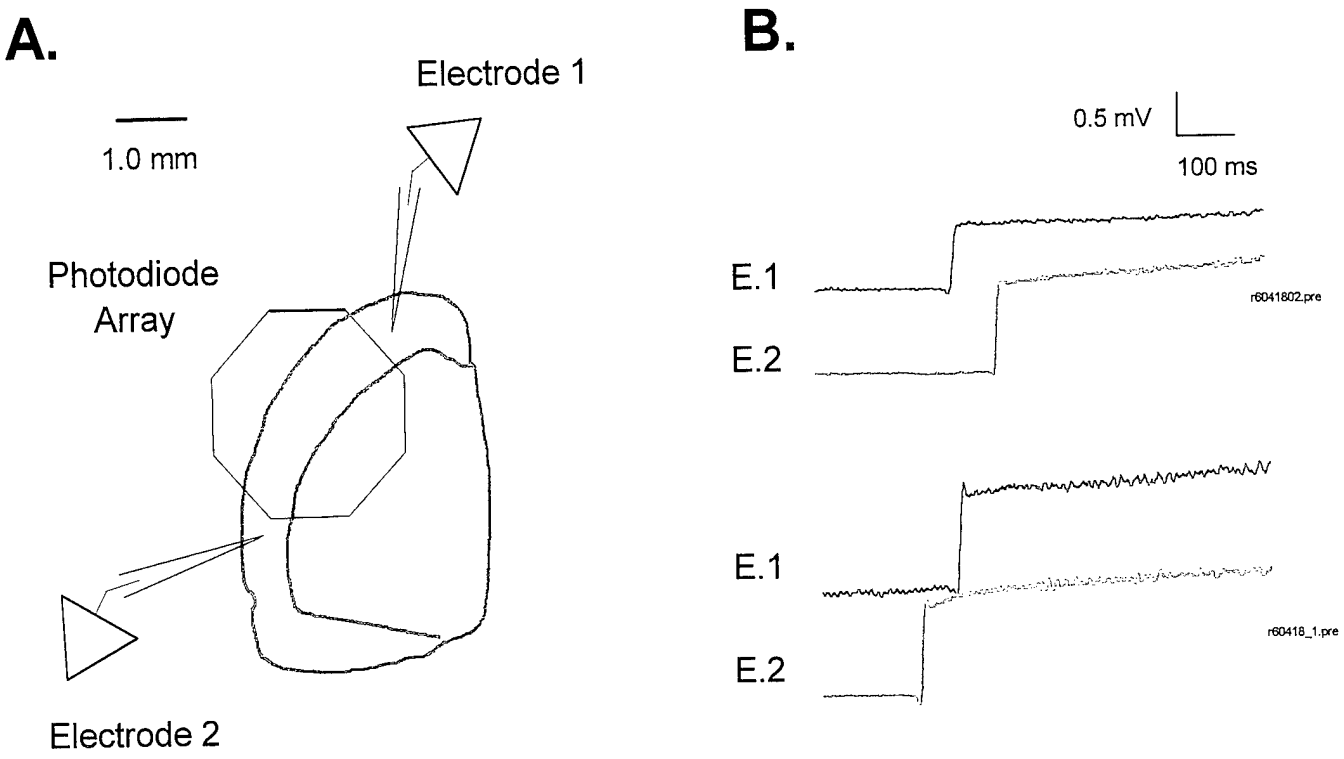
We use a 124-channel parallel diode array system to obtain voltage images at a high sampling rate (ca., 0.5 ms per frame), fast enough to monitor the initiation and propagation of epileptiform events in neocortex slice preparations. We have visualized spontaneous PDS (Figure 2--Appendix) in single trials without signal averaging. This allows us to locate the initiation site and study its dynamic changes in individual trials. We have also obtained images of intrinsic optical signals and can continuously monitor thousands of ictal-like events. Voltage sensitive dye signals are directly proportional to the transmembrane potentials of the neurons rather than to the current, as measured by electrodes. The dye image is thus not biased by the current source and sink in the brain structures, so the initiation site (s) of synchronized global events can be accurately examined. In this proposal we intend to take advantage of speed and accuracy afforded by this new approach to test a number of hypothetical mechanisms describing how apparent local activities develop into synchronized epoch discharges.

Results: PDS as a propagation wave

Neocortical slices developed spontaneous epochs featured by an all-or none initial event followed by cycles of oscillation. The initial event or paroxysmal depolarize shift, (PDS) is a long lasting large event. The voltage sensitive imaging showed that the initial event is a slow propagation wave with a sharp depolarizing phase and a long lasting hyperpolarizing phase. Unit recordings revealed that neurons fire at a high rate during the initial wave.

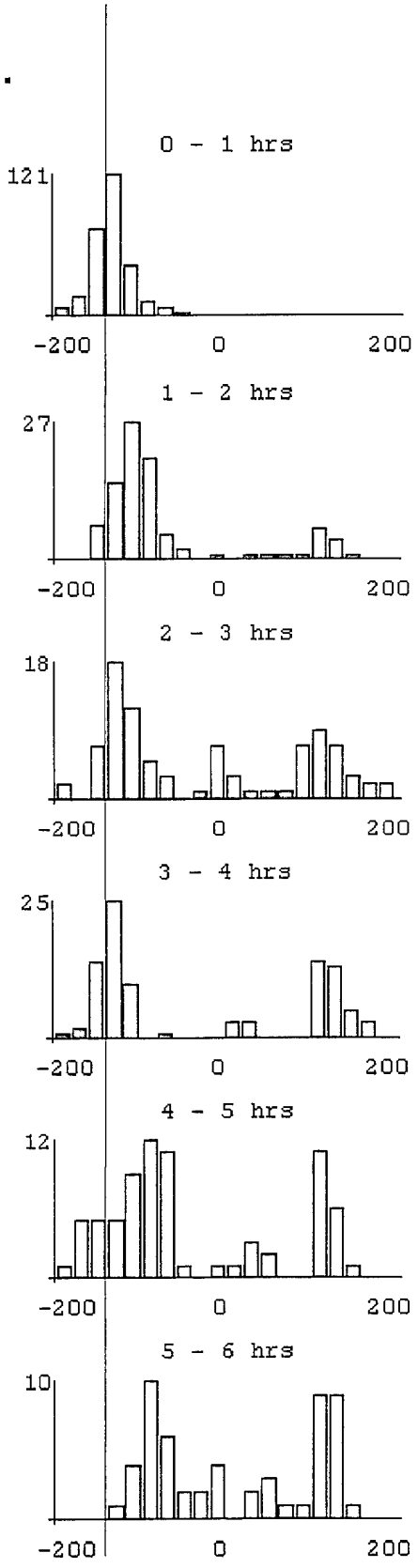
The initial event propagates over the entire preparation. The propagation pattern of the initial wave is very complex. Pseudo color movie displays showed that even in a small cortical section (the field of view of 1.2 mm in diameter), one can see turbulence and sharp turns in the preparation pattern. On the top row of Figure 2 and Figure 3 C are selected time series of images of the PDS propagation. Unfortunately in this multi-frame display the dynamics in propagation can not be faithfully reproduced. We are unable to submit a disk with our pseudo-color movie display as an appendix because our program and data files are very large and require IDL to run . However, upon request, we will try to write a specialized display program packed with a data file on a single CD for examination. The propagation of the initial event can also be measured by electrode recordings. (Figure 4) The propagation velocity of the initial wave (as

ELECTRICAL AND OPTICAL RECORDINGS 3



INITIATION SITES OF PDS EVENTS

A.



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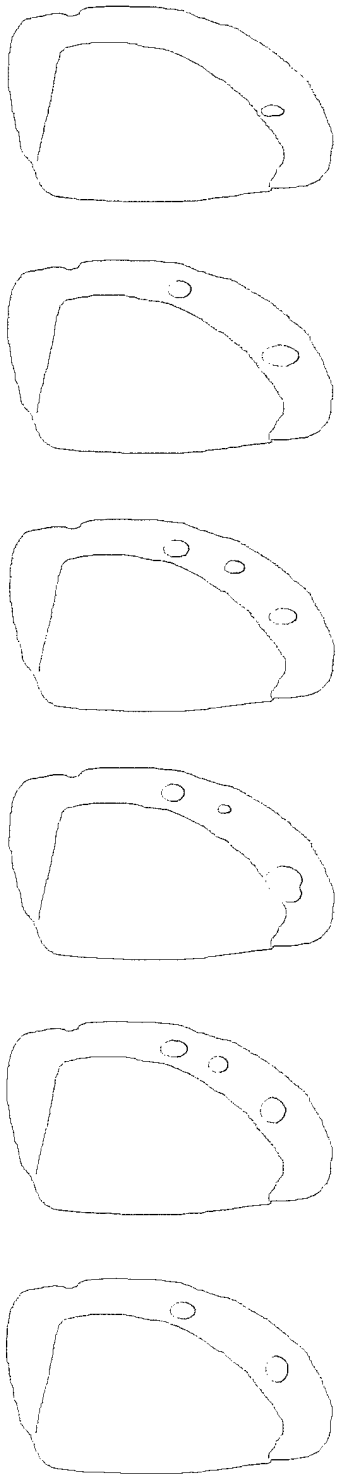
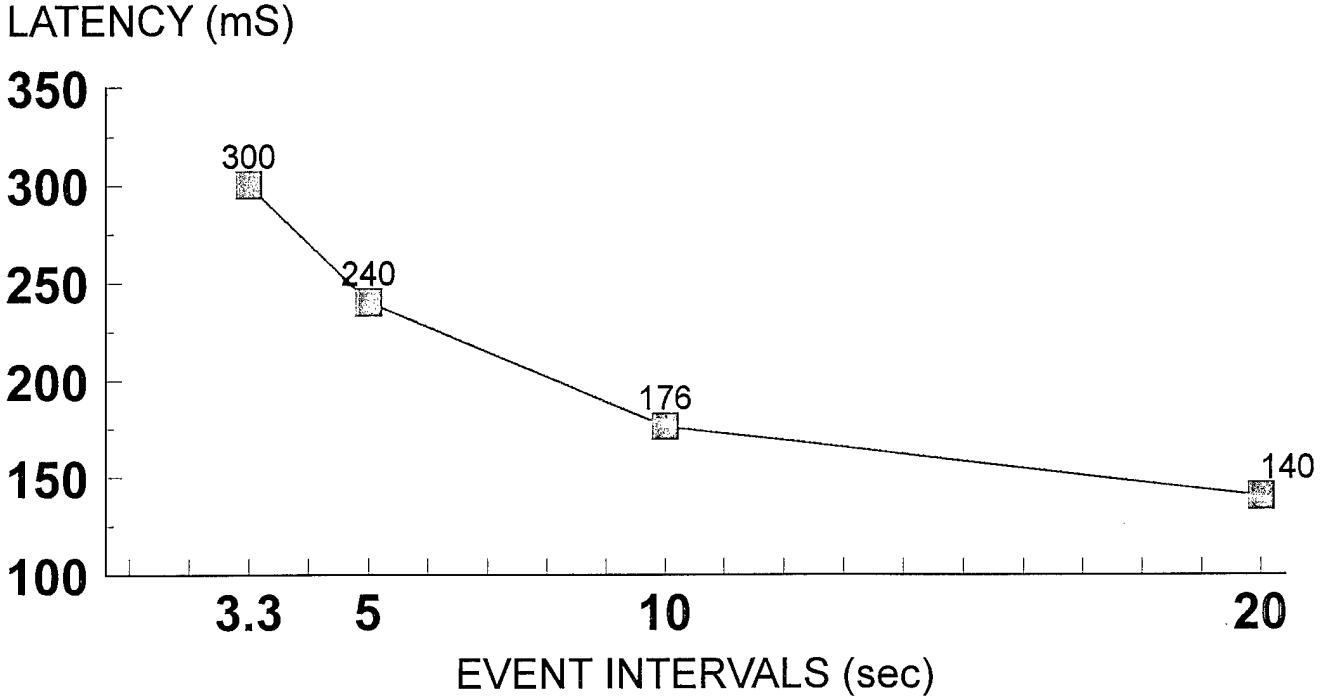
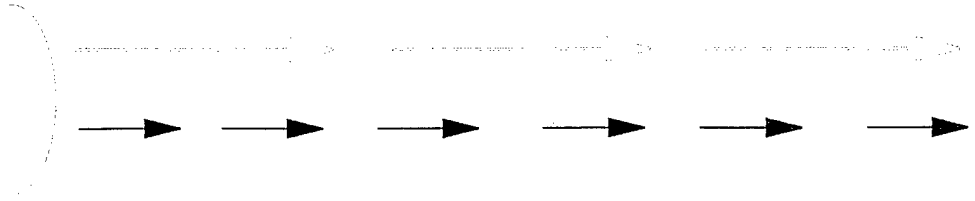


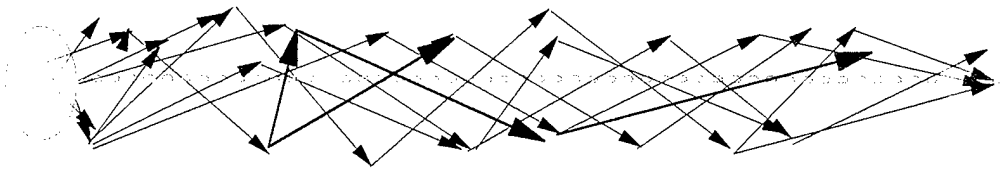
RELATIONSHIP BETWEEN EVENT INTERVALS AND LATENCIES



The Velocity Model



The Path Model



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measured by the two electrodes) appeared slow and varied substantially from epoch to epoch. This suggested that the propagation of the PDS involves a large number of synapses which make the actual propagation path complex and uncertain (cf. Figure 5, the path model).

Results: Potentiation of the PDS initiation site and propagation path

In our experiments recordings could last more than 10 hours, with the preparation still appearing healthy with vigorous spontaneous oscillations. This made it possible to study activity-related plasticity. The main question is whether the spontaneous epoch PDS and oscillations, as one kind of activity, would modify the local circuitry and then alter the spontaneous event. The activity dependent modifications to the neural circuitry can be seen from two aspects: The potentiation of the initiation site and the potentiation of the propagation path.

Figure 3C is a selected set of a time series of voltage sensitive dye images of a pseudo color movie. In the top row the PDS event initiated from the top of the field of view. In another event started later on the same field of view, it started from the bottom of the view. In theory in one slice preparation there could be many initiation sites for the spontaneous PDS events. However, in our experiments we see only a few dominate initiation sites which start most of the events (Figure 4). This suggested that there is a certain kind of competitive inhibition. We continuously monitor the changes in the location of the initiation site (Figure 3B), as indicated by the time delay between two recording electrodes (Figure 4A). During the first few hours after the spontaneous event starts, there is only one initiation center on the slice and then the second and third develop. We are designing new experiments to further study the development of an initiation site.

The propagation path of the PDS events also shows some extent of potentiation. The apparent propagation velocity became higher after hundreds of spontaneous events. This suggested that the propagation path is optimized by ongoing activity. Interestingly, if the time between two spontaneous events is shorter than 5 min, the propagation velocity is much slower for the second event (Figure 5). This suggests that synaptic fatigue may alter the propagation path (the path model of Figure 5) or alter the synaptic efficiency (the velocity model of Figure 5). In either case the propagation of the PDS must involve a huge number of synapses, and a large portion of them undergo potentiation during the spontaneous epoch events.

Results: Intrinsic optical signals: Different from neural activity

The intrinsic optical signals during the PDS events are about 10 times larger than the voltage signals. Intrinsic signals have a slow raising phase, take a few seconds to reach their peak after the peak of the voltage sensitive dye signal and last about 5 seconds after a single PDS event. The intrinsic signals seemed to start from cortical layers II and III and later move to deep layers. This has never been reported. We also found that intrinsic optical signals are a non-linear function of the activity. In some active level the signal intensity is not proportional to the activity level. This has never been noticed by other researchers. Since intrinsic optical measurement attracts more and more attention recently as a tool for monitoring brain activity. With our cooled CCD camera, we have been able to obtain a high resolution image of the intrinsic optic signals. A 12 bit signal depth of the camera gave us 16 gray levels of the intensity structure. We cannot present intrinsic images here since we are still working on the software that can present the CCD camera images.

Conclusions

The long term objective of this research is to understand the initiation of synchronized epileptiform events. The recent application of optical recordings using voltage sensitive dyes has created new possibilities for studying spatio-temporal dynamics during epileptiform activities. We utilize voltage sensitive dyes and intrinsic optical signal imaging techniques to visualize the initiation of all-or-none paroxysmal depolarizing shift (PDS) and synchronized ictal-like events in rat cortical slices. Imaging electrical activity allows us to directly test several hypotheses about how synchronized events start from localized neural activity. Data showed that we were able to pin-point the initiation sites of synchronized events with a high temporal resolution (0.5 ms per frame) and to continuously monitor the dynamic changes in the spontaneous events for a period of 8 hours (about 1,500 spontaneous events). We found that PDS

events are started from deep cortical layers and spread horizontally at about 40 mm/sec. On one slice preparation only a few initiation sites dominated the initiation of the PDS events. The propagation speed of the PDS events became faster when more spontaneous events took place. It seemed that the propagation path underwent a potentiation process which either shortened the path or increased the propagation velocity. Part of the data has been submitted as preliminary result for an application for an Epilepsy Foundation grant. We are writing a paper for the publication of these results.

Project 3: Collaboration Project

Introduction

In collaboration with Dr. Samuel Rabkin of the Department of Neurosurgery, we have begun a project in which gene expression is visualized in real time by optical recording following delivery of transgenes to the CNS. The long term goal of this collaborative project is to examine the functional consequences on cortical circuitry of genetically altering the neurotransmitter balance at excitatory synapses in adult primary sensory cortex. The specific aim is to functionally assess whether viral delivery of engineered genes leads to functional alterations in vivo in an animal system. Cells in the cerebral cortex will be infected with a defective herpes simplex virus (HSV) vector containing the transgene for glutamic acid decarboxylase (GAD), an enzyme that converts glutamate, an excitatory neurotransmitter, to GABA, an inhibitory neurotransmitter. The impact on synaptic activity of increasing the amount of GABA at excitatory synapses will be determined by high speed optical imaging of voltage-sensitive dye signals in cortical slices injected with HSV vector. Gene expression will also be confirmed by histochemistry for detection of lac Z, a reporter gene delivered along with the GAD transgene. Defective HSV vectors have been used successfully to deliver the GAD transgene to cells of the central nervous system (CNS). Optical recording is an extremely sensitive method to determine the synaptic activity of a large number of neurons in a neural circuit in real time, both in vitro and in vivo. We have combined the technical capabilities present in the participating laboratories, viral vectors for gene delivery and optical recording, in a unique approach that will allow visualization of the consequences of GAD transgene expression in the CNS.

Significance: Neurotransmitter imbalance is a concern of great importance in many disease states, as well as being essential to the understanding of normal synaptic function. Currently, there is an enormous amount of interest in gene delivery to the CNS to ameliorate or alleviate conditions brought on by transmitter imbalance. However, as yet there are no functional models to demonstrate the efficacy and viability of gene delivery to the CNS. With this project, we plan to determine the viability of using gene delivery systems in animal models, and hope to extend the technique to slices of human cortex.

Results: Lac gene expression

In the initial phase of the experiment, it was essential to ascertain the practicability of our methods, and to determine the appropriate vectors to test gene expression in cortical slices maintained in vitro. Thus, we focused primarily on histochemical analysis on cortical slices injected with viral vector. Coronal slices of cerebral cortex, 400 mm thick, were obtained from 3-week-old rats. Small volumes of virus (200-400 ml) were injected into several locations within layers 2, 3 or 4. After a period of time sufficient to allow expression, the slices were fixed and processed for B-galactosidase histochemistry for detection of lac Z expression. Positive staining was obtained in four slices injected with vector bearing the reporter gene lac Z. Experiments are currently underway for testing the GAD vector and for optical imaging.

COMPUTATIONAL NEUROSCIENCE: There are currently two faculty members in this area. Dr. Goodhill's research relates to theoretical analysis of the development and pattern of connections in the brain. Dr. Pouget focuses on computational modeling of neural representations.

GEOFFREY GOODHILL, PH.D.

Project 1

The focus of the work is on models of brain development. Biological nervous systems have a remarkable capacity to self-organize into highly effective computational structures. They do this in response to a mixture of genetically programmed instructions and signals from the external world. Understanding the rules that govern these processes in biological systems will help in the design of more sophisticated artificial computing devices, and in understanding how the brain can be encouraged to repair itself after injury.

There are two main projects being pursued. The first addresses the development and structure of topographic maps in the cortex. Previously, he predicted that the periodicity of the ocular dominance column map in primary visual cortex is partly determined by the structure of correlations in the visual input (Goodhill and Willshaw, 1990, 1994; Goodhill, 1993), a prediction that was subsequently confirmed experimentally (Goodhill and Lowel, 1995). It has also been shown theoretically that the characteristic global structure of this map may be influenced by visual activity (Goodhill, Bates and Montague, 1996). This prediction has yet to be tested. More recently, Dr. Goodhill has begun to investigate the abstract optimization principles that may underlie topographic map structure (Goodhill and Sejnowski, 1996). The goal is now to use these principles to learn more about the computational capabilities of maps throughout the cortex (Goodhill, Finch and Sejnowski, 1996).

Project 2

The second project addresses how axons are guided to appropriate targets in the developing brain. Many experiments have shown that gradients of chemoattractant and repellent factors play an important role. However, there are no computational theories for how axonal growth cones actually sense and are guided by such gradients. Recent work by Dr. Goodhill has investigated the spatial limits over which axons could conceivably be guided by a gradient (Goodhill and Baier, 1996). He showed that this limit is about 1cm for an optimal gradient, and 1mm for a gradient of a target-derived factor set up by diffusion (Goodhill, 1996). These theoretical limits fit well with what is known experimentally. Future work will model more directly the process of receptor binding, and how this is converted into a guidance signal.

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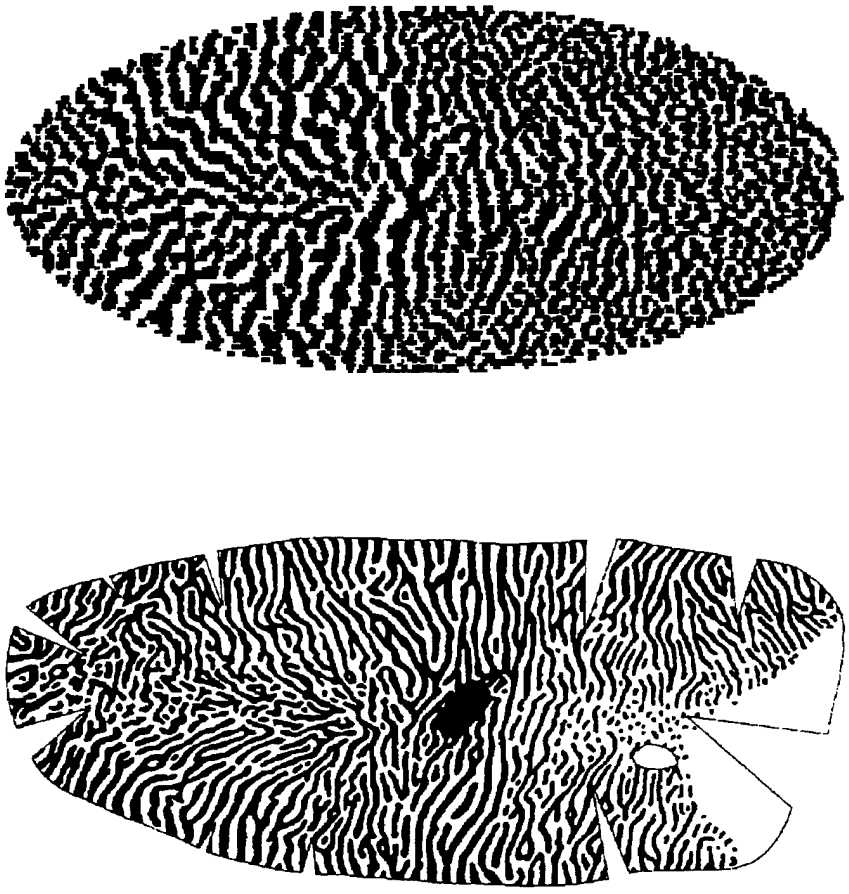

Figure 1: The complete pattern of ocular dominance columns in a monkey compared to the output of a computational model. The top picture was reconstructed from serial sections of macaque primary visual cortex by Simon LeVay and colleagues. The bottom picture was generated using the elastic net model for ocular dominance column formation (Goodhill, Bates & Montague, submitted). The model captures many features of the pattern in the macaque, including the thickening of columns in the foveal representation. This work yields new insights into how genetic and epigenetic factors interact to determine the structure of cortical maps.

ALEXANDRE POUGET, PH.D.

Theoretical Neuroscience Laboratory

Introduction

The goal of my laboratory is to elucidate the mechanisms of information processing in cortical circuitry. I use primarily a computational approach involving mathematical analysis and computer simulations. My research focuses on how neurons encode sensory information through their firing rate and how these signals are being subsequently used to guide motor behaviors such as grasping or navigation.

Project 1: Neural Coding: Lateral Connections and Noise Filtering

There are many ways information processing systems can encode information. Computers, for example, encode signals with a binary code, i.e., strings of 0's and 1's. In the brain, neurons communicate by sending electrical pulses, or spikes, along their axon. These spikes trigger the release of neurotransmitter at the synapses which, in turn, changes the membrane potential of the post-synaptic neurons. Information is believed to be encoded through the firing rate --- the number of impulses per second --- and possibly the timing of the spikes. Post-synaptic neurons are faced with the problem of having to decode, or interpret, these responses, but little is known about how they perform this task. The difficulty in decoding neural signals comes largely from the presence of large amount of noise in cortical circuitry. Thus, in response to a fixed stimulus, a neuron might fire 3 spikes on average over many trials but it can be 2 spikes or 6 spikes on any particular trial.

We have recently started to work on this problem and found that lateral connections, connections linking neurons at the same hierarchical level of processing, can be used to perform maximum likelihood estimations, a statistically optimum decoding method (Zhang et al., 1996; Pouget & Zhang, 1997a: Pouget & Zhang, 1997b). Our model uses lateral connections to implement expectations about sensory signals. These expectations allow the system to filter out the noise non-linearly while preserving the maximum information about the signal.

We applied this model to the problem of decoding the response of neurons selective to the direction of motion of visual stimuli. The goal was to come up with a guess, or estimate, of the direction of motion of a stimulus on the basis of the noisy response of motion selective neurons. An estimate based on such activity patterns is bound to vary from trial to trial due to the neuronal noise, even if the true direction is kept constant. An efficient estimate must minimize this variability, that is to say, the standard deviation of the estimate must be as small as possible.

Figure 1 shows the standard deviation of the estimate obtained with our model (RN, recurrent network) compared to four classical estimation methods used by neurophysiologists for decoding neuronal activity. Our results show that our method performs as well, or nearly as well, as the maximum likelihood estimator (ML), a procedure which is known to lead to the minimum standard deviation. In other words, our model provides a means by which lateral connections can perform maximum likelihood estimations, which is the optimal way to filter out noise.

This work has important implications for orientation selectivity in the primary visual cortex (V1). Since the work of Hubel and Wiesel, it has been known that cells in V1 are tuned to the orientation of visual contours. Typically, they have a bell-shaped tuning curve to orientation with a half-width (width of the tuning curve at half of the maximum response) of 30-40 degrees. One influential theory posits that the inputs coming from the LGN are poorly tuned (half-width of 90 degrees or more) but are subsequently sharpened through a competition mediated by the lateral connections between cells tuned to different orientations.

We have found, however, that this strategy is statistically suboptimal and would result in important loss of information (Pouget & Zhang, 1996). Our analysis suggests that it would be more efficient to start with pretuned LGN inputs (half-width of 30-40 degrees) and to use lateral connections to filter out noise, a scheme which is supported by recent experimental data from Fertser et al (Nature, 380. 1996).

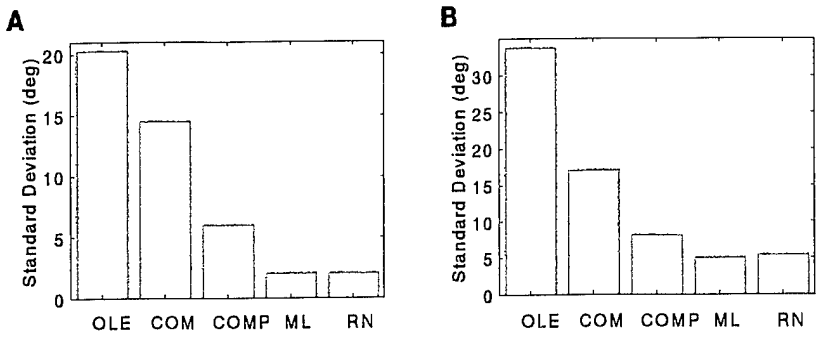

Figure 1: Histogram of the standard deviation of the estimate for four classical methods (OLE, optimal linear estimator; COM, center of mass; COMP, complex estimator; ML, maximum likelihood) and our network using lateral connections (RN, recurrent network). A- Noise with normal distribution. B- Noise with Poisson distribution. In both cases, the network with lateral connections (RN) performs nearly as well as maximum likelihood (ML), a statistically optimum method.

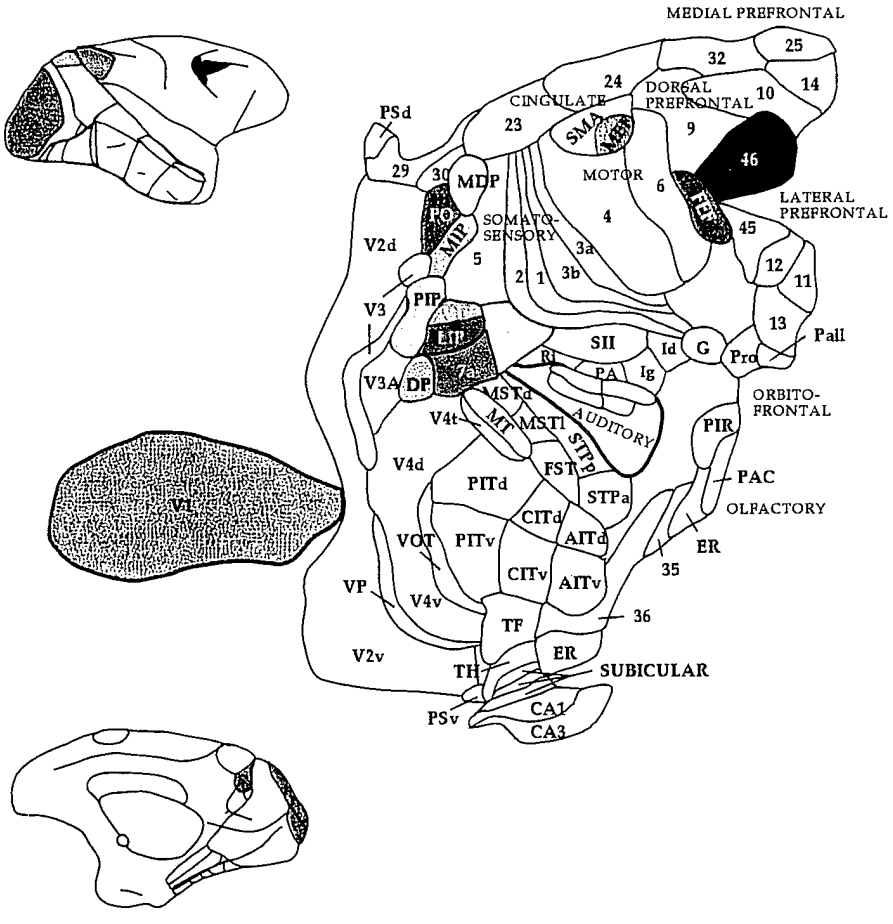

Figure 2: Flattened representation of the cortex of a macaque monkey. Cortical areas with the parietal lobe are highlighted in color (adapted from Felleman and Van Essen Cerebral Cortex, 1991).

In the coming years, we intend to extend this work to multisensory integration, the process by which information pertaining to the same object, but coming from different sensory modalities, such as the face and voice of a person, is merged in a unitary representation. We will explore various statistical strategies as well as plausible neuronal implementations.

Project 2: Basis Function Models of Spatial Representations

The other major line of research in my laboratory deals with spatial perception, the perception of the location of objects in space. In order to grasp an object, it is critical to have some knowledge of the position of the object with respect to oneself. How do neurons encode this information? Most researchers in the field formalize this issue as a geometry problem, i.e., as a problem of specifying the coordinates of the object in a frame of reference centered on the viewer. By contrast, we have adopted a functional approach which considers the problem within the context of sensorimotor transformations.

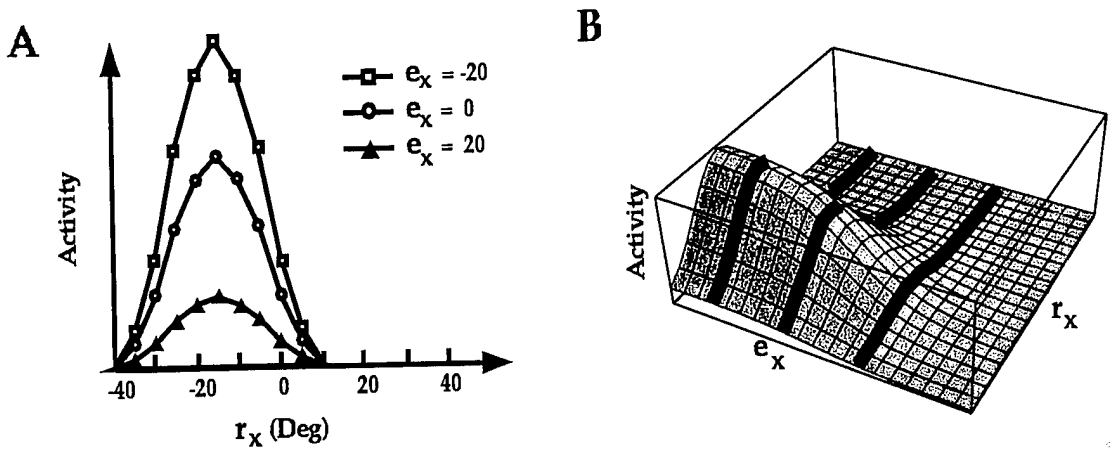

Figure 3: A- Idealization of a retinotopic visual receptive field of a typical parietal neuron for three different gaze angles (e_x). Note that eye position modulates the amplitude of the response but does not affect the retinotopic position of the receptive field (adapted from Andersen et al., Science, 230, 1985). B- 3D plot showing the response function of an idealized parietal neuron for all possible eye and retinotopic positions, e_x and r_x. The plot in *A* was obtained by mapping the visual receptive field of this idealized parietal neuron for three different eye positions as indicated by the bold lines.

Our theory is based on the idea that the brain uses representations which simplify the computations that it needs to perform. In general, the choice of a representation strongly constrains whether a particular computation will be easy or not. Hence, addition is easy in our decimal system but impossible with Roman numerals. The same is true for spatial representations, some codes make it easier to compute motor commands such as grasping.

Using this computational constraint, we have developed a theoretical framework (Pouget & Sejnowski, 1996b) --- based on the theory of basis functions --- to interpret the response of single cells in the parietal cortex, the part of the brain involved in spatial perception (figure 2). More specifically, we proposed that parietal neurons compute basis functions of their sensory inputs, i.e., a set of building blocks from which any function, and therefore any motor command, can be easily generated (figure 3).

Our claim is based on the properties of the visual receptive field of parietal neurons. The receptive field of most parietal cells is fixed on the retina, as for V1 neurons. The amplitude of their response to a light, however, is modulated by eye position (Andersen et al., Science, 230, 1985): typically, the response to a visual stimulus in the center of the receptive field increases monotonically as the eye moves along a particular direction in space, specific to each cell (see figure 3-A). This response can be described as the product of a gaussian function of retinal location multiplied by a sigmoid function of eye position (Fig. 3-B). Sets of both Gaussians and sigmoids are basis functions, and the set of all products of these two basis functions also forms basis functions over the joint space.

We are now using this formalization to understand the behavior of human patients with hemineglect, a neurological syndrome associated with parietal damage. This syndrome is characterized by an inability to respond to and process stimuli presented in the contralesional hemispace. For instance, a patient with right parietal damage will typically fail to eat the food located on the left side of his plate, or will forget to dress on the left side of his body. In some cases, the syndrome becomes so extreme that the patient can simply deny that his left arm and leg belong to him. We have recently demonstrated that our basis function approach can account for the behavior of these patients in a wide variety of tasks (Pouget and Sejnowski, 1996a; Pouget and Sejnowski, in press 1997) thereby relating the syndrome to its neural basis.

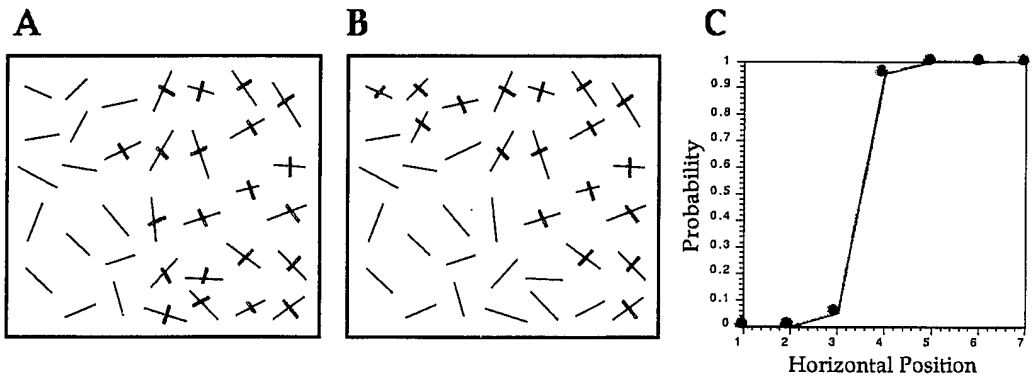

Figure 4: A- Typical crossing pattern for the network in line cancellation experiments after a right parietal lesion. The network misses the lines on the left side just like left neglect patients. B- Same as in A, but with a lesion which introduces an oblique retinal gradient, up and right. The network misses the lines in the bottom left half of the display. Such diagonal gradients have also been reported in some patients. C- probability of a line to be crossed out as a function of its position on the display for a horizontal retinal gradient as in A. The step function profile of this curve and the fact that it saturates at zero on the left side explains why the network behaves as if it were completely blind to bars located on the left side of the display.

Figure 4, for example, shows how our lesioned model performs on a line cancellation task, a test in which patients are asked to cross out short line segments uniformly spread over a page. Left neglect patients, that is to say patients with a right parietal lesion, typically fail to check the line segments in the left half of the display. We observed the same behavior in our model after simulating a right hemisphere lesion.

We are also currently investigating how the model can recover from a unilateral lesion. We have identified three distinct recovery processes which we intend to characterize further in the coming year. We believe that this study will shed light on the mechanism involved in the recovery in neglect patients and could potentially have important implications for rehabilitation.

Future Directions

This work illustrates the long term goal of our laboratory: to relate the response of single neurons to cognitive behaviors. We believe that this goal can be achieved only through strong interactions between experimentalists and modelers. Accordingly, our research is conducted in close collaboration with experimentalists from the Georgetown Institute of Cognitive and Computational Sciences as well as other institutions.

We are presently in the process of developing an experimental set up for testing hemineglect patients. This is done in collaboration with Jorge Kattah in the Neurology department of the Georgetown University Medical School. This set up will allow us to present complex visual stimuli and to measure eye movements with high resolution using the search coil techniques. Our goal is to test some of the predictions that have emerged from our modeling work. Mickey Goldberg (from NIH), a leader in the neurophysiology of the parietal cortex, is also involved in this project, as well as John McClurkin (NIH) who will be

providing technical support. We expect to have a working set up in the Winter and to start testing patients by the Spring.

I am also starting a collaboration with Jon Driver from University of London. Dr. Driver has published several important papers on how hemineglect interacts with visual segmentation and he has recently started to investigate multisensory integration in neglect patients. We are planning to continue this work on multisensory integration together, a project which will be tightly linked to our computational modeling on the subject.

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DRUG DISCOVERY AND DESIGN: There are currently two faculty members in this area. Dr. Kozikowski's work relates to the design and synthesis of new pharmacological research tools for understanding brain mechanisms, including cognitive drug development. Dr. Wang utilizes molecular modeling techniques as part of drug development.

ALAN P. KOZIKOWSKI. PH.D.

Project 1: Development of novel drugs and pharmacological tools for probing the mechanisms of cognitive dysfunction and cellular signaling

Introduction

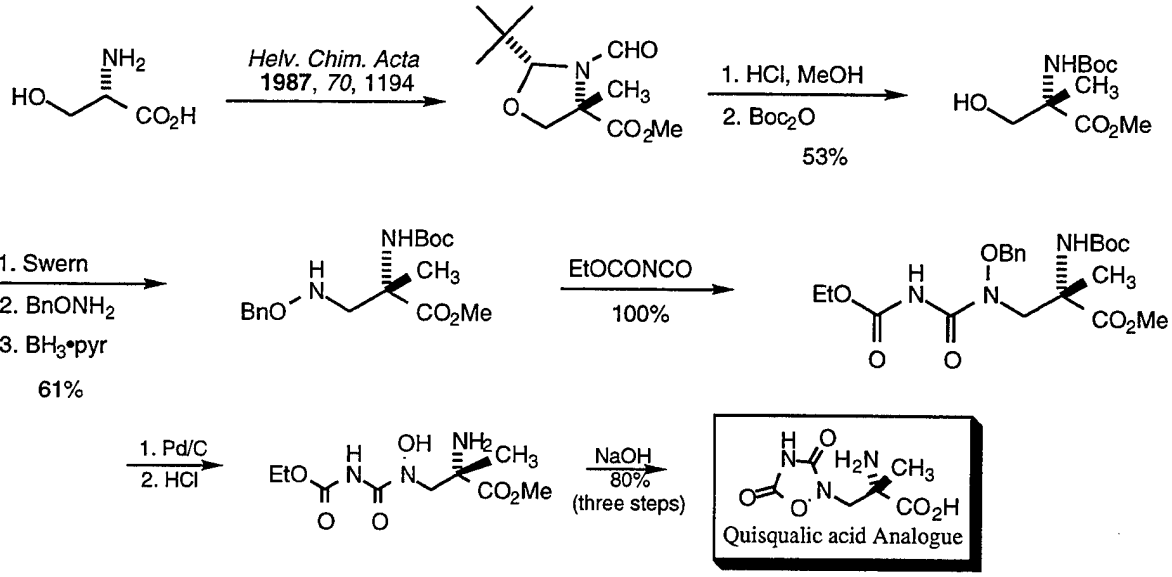
Dr. Kozikowski's group is actively involved in the design and synthesis of new molecular entities for use in probing various aspects of brain function. Such work demands a multidisciplinary setting, in which modern day methods of organic synthesis are coupled with state-of-the-art molecular modeling capabilities, and with cutting edge biology. While details of the various projects are delineated below, we note here that one of the most exciting projects relates to huperzine A. It is our intention to advance huperzine A or one of its more potent analogues to the clinic for the treatment of Alzheimer's disease.

Development of the Laboratory: During the past year a drug discovery group has been assembled within GICCS. A total of five postdoctoral fellows trained in the art of organic synthesis are presently working in the Kozikowski laboratories, and a sixth fellow is now being recruited. The laboratory has been outfitted with chemical hoods in order to accommodate this chemical synthesis/drug design activity. Moreover, a state-of-the-art high field NMR is now in place which is essential to adequately characterizing all new compounds synthesized in our laboratories. Other spectroscopic tools available for our work include FT-IR, low resolution GC-MS, and polarimeter.

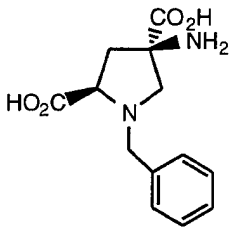
Furthermore, the drug design program has been strengthened through the recruitment of Dr. Shaomeng Wang, an Assistant Professor skilled in the art of computer modeling. Dr. Wang's expertise makes it possible for us to view the target proteins on the computer, especially in the cases where x-ray structural information is available, and to carry out systematic docking of small molecules to the macromolecular targets. Such docking studies allow for the more rational design of small molecules likely to exhibit the desired pharmacological properties.

Project 1: Metabotropic Glutamate Analogues

Specifically, in a project relating to our interest in elucidating the role of metabotropic glutamate receptor (mGluR) subtypes in normal and pathophysiological states, we have undertaken the design, synthesis and biological assay of compounds that may exhibit selectivity for the individual mGluRs. To date we have discovered that derivatization of quisqualic acid leads to a mGluR2 specific antagonist, and that the N-benzylation of the aza analogue of ACPD leads to an mGluR6 selective agonist. The latter compound was found to be neuroprotective in cellular assays of brain injury. Using various lead structures that are now available to us, it is our intention to expand the present effort using molecular modeling techniques so as to acquire a better understanding of the topography, both steric and electronic, of the individual mGluRs thus aiding the design of compounds of altered subtype selectivity, potency, and degrees of agonism or antagonism. This research may find eventual application in the discovery of ligands of clinical use in the treatment of Alzheimer's disease, stroke, spinal cord injury, and Parkinson's disease.

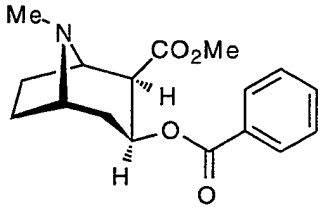


2(*S*)-2-Amino-2-methyl-3-[2'-(3',5'-dioxo-1',2',4'-oxadiazolidinyl)]-propanoic Acid (AMOP)

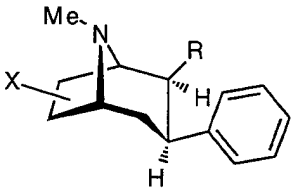


1-Benzyl Analogue of Aza-ACPD

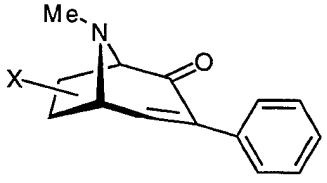
We have now worked out methods for gaining access to a wide variety of analogues of cocaine using a dipolar cycloaddition approach of oxidopyridinium salt with dipolarophile. This novel approach allows access to a wide variety of cocaine analogues of Type I bearing diverse functionality at positions 1, 2, 3, 6, and 7 by way of the tropenone of Type II. Previously, we had found that methoxylation of cocaine's 6-position led to a compound that acts as a weak cocaine antagonist. The present chemistry thus permits us to expand upon the past work, and to now explore whether it is possible to retain such "antagonistic" activity while at the same time increasing compound potency. The identification of a cocaine antagonist or cocaine partial agonist may find use in the treatment of cocaine abuse.



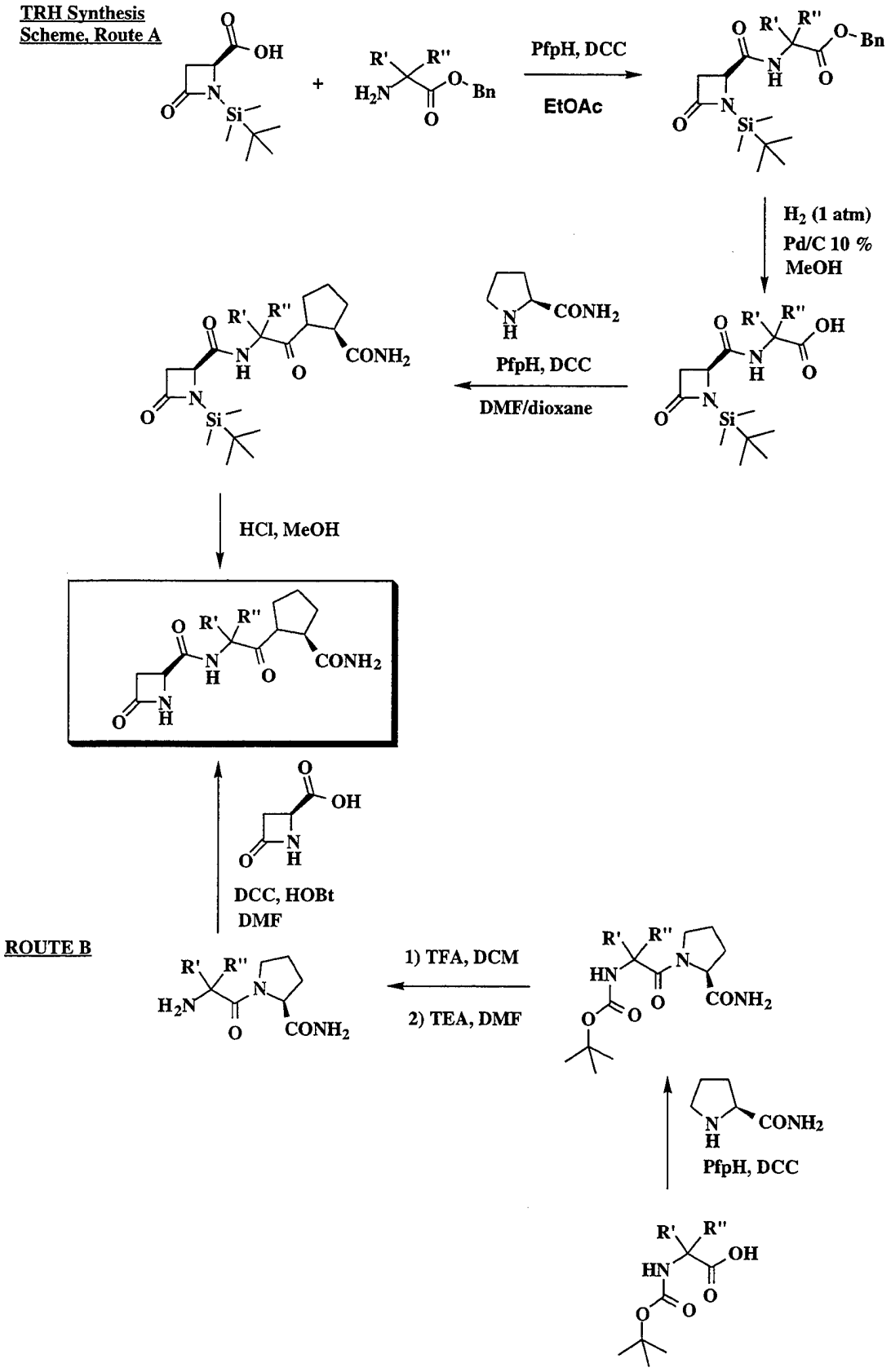
Cocaine



3-Phenyltropane of Type I



3-Phenyltropen-2-one of Type II


TRH Synthesis
Scheme, Route A
PfpH, DCC
EtOAc
H2 (1 atm)
Pd/C 10 %
MeOH
PfpH, DCC
DMF/dioxane
HCl, MeOH
DCC, HOBt
DMF
ROUTE B
1) TFA, DCM
2) TEA, DMF
PfpH, DCC

We have synthesized a number of novel structural analogues of thyrotropin-releasing hormone (TRH) that may find use in traumatic brain injury (see accompanying TRH synthesis scheme). A variety of TRH analogues in which its histidine moiety has been replaced by functionality believed to enhance its neuroprotective activity have now been assembled in our laboratories. The new analogues are now under active study in the laboratories of Dr. Faden, whose research team is examining the effects of these compounds in animal models of brain injury.

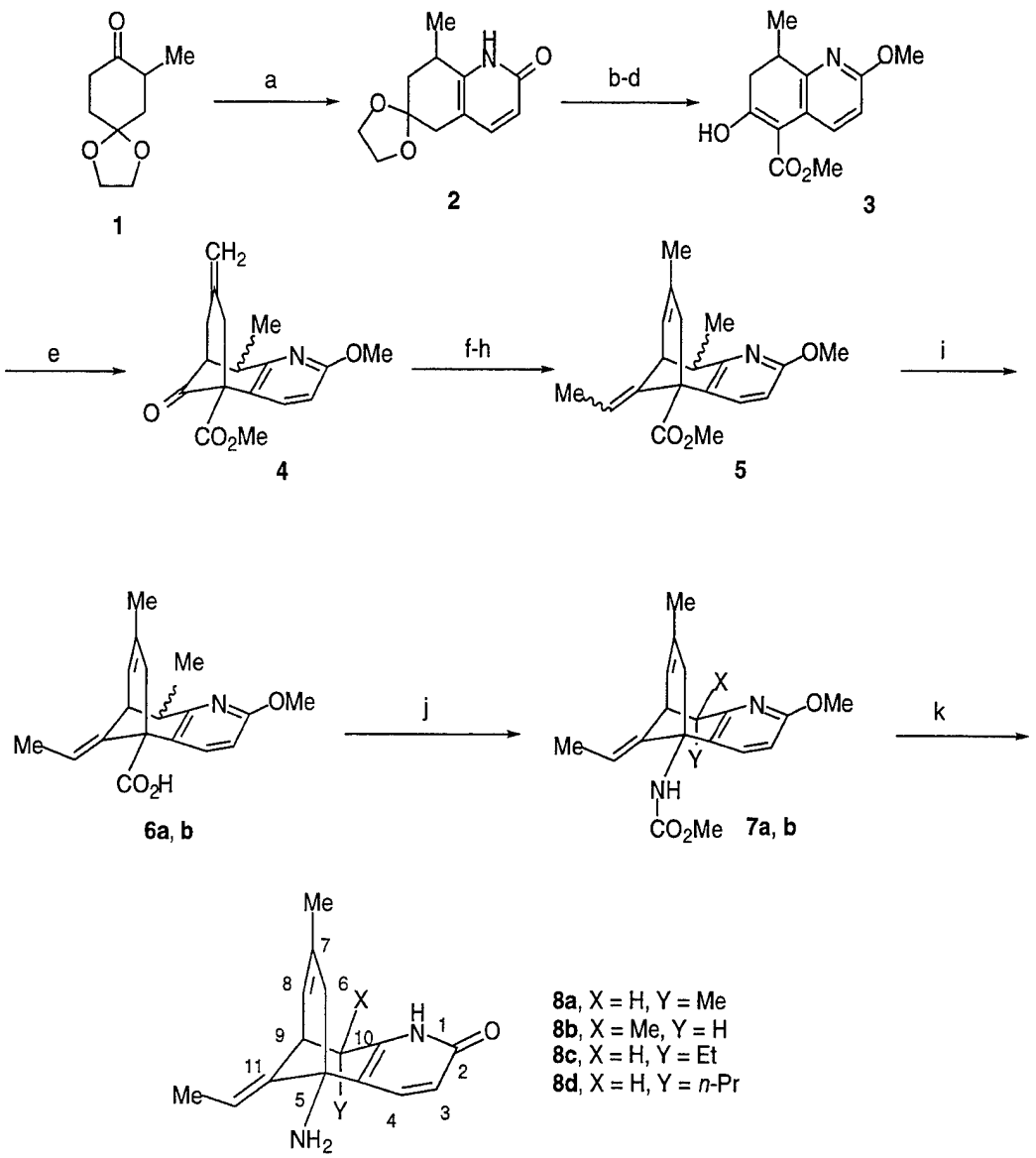
We have conducted extensive modeling studies on huperzine A, a naturally occurring alkaloid that has shown promise in the treatment of Alzheimer's disease. Through the modeling studies we have now identified compounds that may have an increased potency for inhibiting acetylcholinesterase. The modeling studies make use of a co-crystal x-ray structure of HA in complex with AChE. By placing analogues into the same site as that occupied by HA in the crystal, and performing dynamics simulations, we are able to estimate whether the new analogues are likely to serve as improved inhibitors of the enzyme. For example, using the modeling techniques, we have been able to explain why the location of a methyl group at HA's C-10 position leads to a compound exhibiting 8-fold improved potency, whereas introduction of a C-10 equatorial methyl leads to a compound possessing a higher K_I. In the former case the methyl group points into a hydrophobic region, whereas in the latter, the methyl group points into a more polar pocket. As the size of the C-10 substituent is increased from methyl to ethyl and propyl, activity drops off dramatically, possibly indicating a serious steric interaction of this substituent with the "walls" of the active site gorge. Clearly, while some additional steric volume is available in the region of the AChE binding site occupied by the C-10 axial substituent of HA, the available space is small, with the methyl analogue serving as the optimal structure. Additionally, as is evident from the BChE activity that is presented, the methyl analogues of HA all retain their high specificity for AChE versus BChE, and, in fact, the axial methyl compound shows an improved selectivity ratio of 2000-fold relative to the 1000-fold selectivity exhibited by HA itself. The AChE versus BChE selectivity may be relevant to minimizing the peripheral effects of such agents in patients. Using such modeling studies, we have now selected additional compounds for synthesis that are likely to show improved activity. By identifying compounds that show altered AChE inhibitory potency, lipophilicity, improved duration of action, etc. it is likely we will be able to identify superior AChE inhibitors for the treatment of Alzheimer's disease.

Table 1. K_I values for the inhibition of FBS AChE and equine BChE by HA and its C-10 Analogues.

Compound	K_I (μM) for FBS AChE[a]	K_I (μM) for Equine BChE[a]
(±)-Huperzine A	0.024	24
Axial methyl 8a	0.003	5.8
Eq. methyl 8b	0.035	5.5
Ethyl 8c	2.04	>100
n-Propyl 8d	>200	>200
(±)-10,10-Dimethylhuperzine A	0.017	9.5

[a]K_I is mean ± standard deviation; standard errors were all within 10% of the mean.

Scheme 1. Synthesis of the 10-Methyl Derivatives **8a** and **8b.**



Reagents: a) NH_3, MeOH, methyl propiolate, 135 °C, 12 h (57%); b) Ag_2CO_3, MeI, $CHCl_3$, 87%; c) 5% HCl, acetone (96%); d) KH, $(MeO)_2CO$, (83%); e) $Pd(OAc)_2$, Ph_3P, DBU, 2-methylene-1,3-propanediol diacetate (89%); f) $EtPPh_3Br$, *n*-BuLi (79%); g) CF_3SO_3H, dioxane, 93 °C (91%); h) PhSH, AIBN, toluene, 85 °C (88%, E/Z = 95:5); i) 20% NaOH, MeOH, THF (83%); j) $(PhO)_2P(O)N_3$, Et_3N, toluene, 85 °C (82%); k) TMSI, $CHCl_3$ (88%).

We have succeeded to synthesize a new protein kinase C (PKC) activator based upon the natural product lead structure, indolactam V (ILV). PKC plays an instrumental role in a variety of intracellular signaling processes, controlling diverse processes such as memory formation and cellular growth. In order to better elucidate the involvement of PKC in cellular signaling, it is mandatory to identify PKC ligands that show enhanced levels of isozyme selectivity, since at present at least 11 isoforms of PKC have been characterized. Based upon extensive modeling studies using the crystal structure information obtained for the cys2 activator binding domain of PKCd, we have designed and synthesized a new ILV ligand, an 8-membered ring benzolactam (Scheme 2, compound 5), that shows enhanced levels of isozyme selectivity, with increased selectivity for the a isozyme of PKC relative to e (Table 2). The difference in the case of the

benzolactam is ten-fold, while the selectivity difference in the case of n-octy-ILV is less than two-fold for the same isozymes.

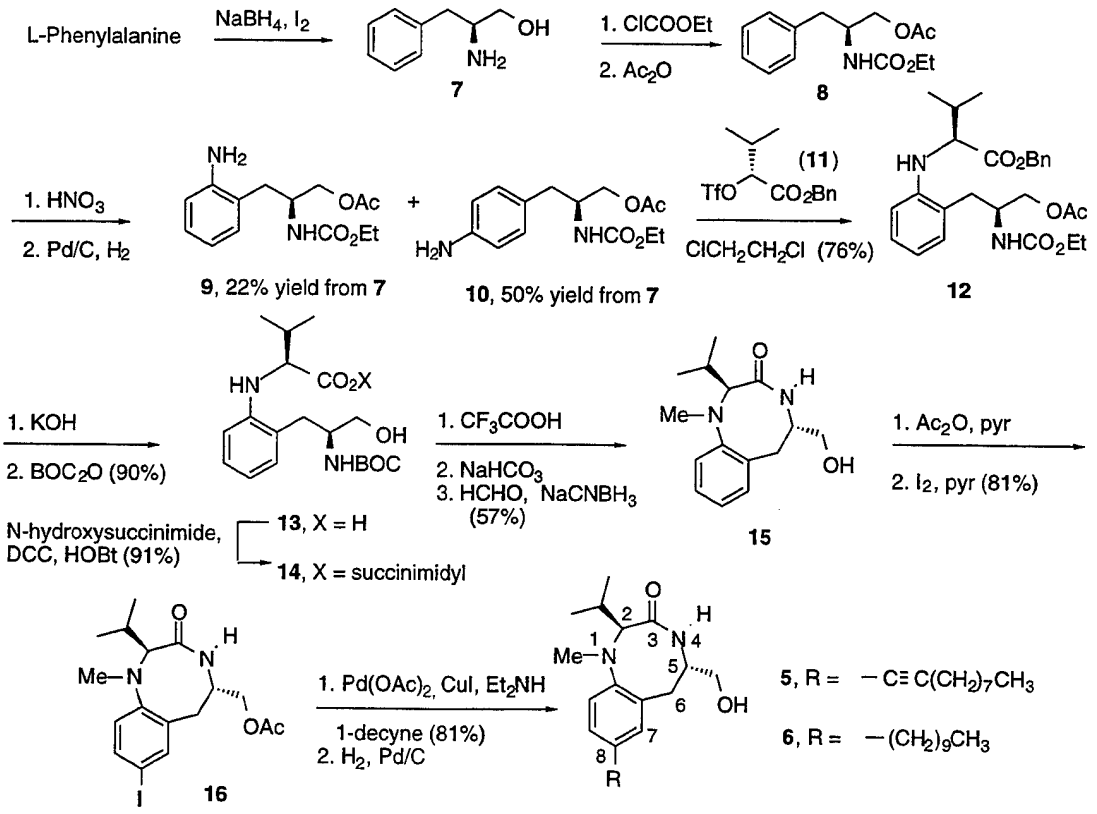
The acetylenic benzolactam 5 and ILV were tested for antiproliferative activity against breast carcinoma cell lines MCF-7 and MDA-MB-231 (Figure 1). Exposure of MCF-7 and MDA-MB-231 cells to 5 for four days resulted in IC_{50} values of 20 and 30 μM, respectively, whereas ILV was inactive. This result is encouraging, and suggests that the benzolactam analogues may find use in cancer chemotherapy.

Interestingly, using this new ligand, Dr. Etcheberrigaray's laboratory has been able to restore the function of silent potassium channels in Alzheimer's fibroblasts. Thus, compounds of the present type may also represent a new approach to the treatment of AD.

Table 2. K_i values ± SEM for the inhibition of [^{3}H]PBDU binding by the compounds tested.

	a	b	g	d	e
Compound					
5	14.7 ± 1.3	17.4 ± 2.2	40.7 ± 8.9	122 ± 22	142 ± 3
6	46.6 ± 8.0	58.2 ± 12.6	145 ± 25	185 ± 30	187 ± 22
ILV*	11.0	6.1	19.4	8.2	21.9
n-octyl-ILV*	0.5	0.4	1.2	0.8	1.0

Scheme 2. An L-Phenylalanine-Based Route to the Benzolactam Analogue of ILV.



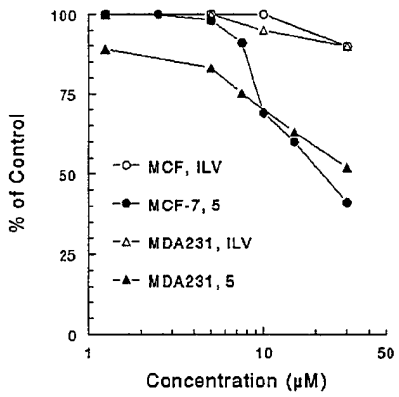


Figure 1: Antiproliferative activity of benzolactam 5 and ILV against two breast carcinoma cell lines.

Conclusions

In conclusion, a number of useful chemical entities have been discovered that may serve as novel pharmacological tools for further elucidiating important aspects of cellular communication. Additionally, compounds have been identified that may actually serve as clinical candidates for the treatment of specific disease processes such as Alzheimer's disease and cancer. It is our plan to continue to work in the promising areas described above in the coming year, with the intention to further refine the activities of these novel agents. Additionally, as time permits, it is our intention to begin efforts to identify novel inhibitors of some of the enzymes involved in programmed cell death, or apoptosis.

SHAOMENG WANG, PH.D.

Project 1: Molecular Modeling of Receptor/Ligand Interactions and Drug Discovery

Introduction

Dr. Wang is interested in the development and use of molecular modeling techniques to study receptor/ligand interactions and the design and discovery of novel therapeutic agents for diseases such as Alzheimer's disease (AD) and cancer.

He has studied a key signal transduction enzyme system, Protein Kinase C (PKC) for the past several years. PKC is a family of enzymes that play a central role in cellular signal transduction, cell growth and differentiation. When he was with the National Cancer Institute (NCI), NIH, He developed a novel, computerized 3D-database pharmacophore searching techniques, which has led to the discovery of a number of classes of novel PKC ligands. He has designed and developed a class of ultra-potent PKC ligands based upon the natural ligand of PKC, diacylglycerol (DAG). These novel, potent PKC ligands provide valuable pharmacological tools for the study of the mechanism of the activation of PKC and characterize their functions in different cell types. Using molecular modeling, his studies have helped to gain much better understanding on how the binding of phorbol esters, DAG and other PKC ligands to the regulatory domain of PKC initiates its activation. Through the studies of a number of classes of PKC ligands, he has shown that even through these high affinity ligands occupy a common binding site on the receptor, they don't share an identical pharmacophore in their structures, a finding could have a major impact to rational drug design. He will continue to pursue his interest in PKC. His current interest in PKC is to predict the three-dimensional regulatory domain structures for all the 11 PKC isozymes, to determine parameters that govern the binding affinity and selectivity of PKC ligands, and to employ structure-based approach for the design and discovery of isozyme selective PKC modulators.

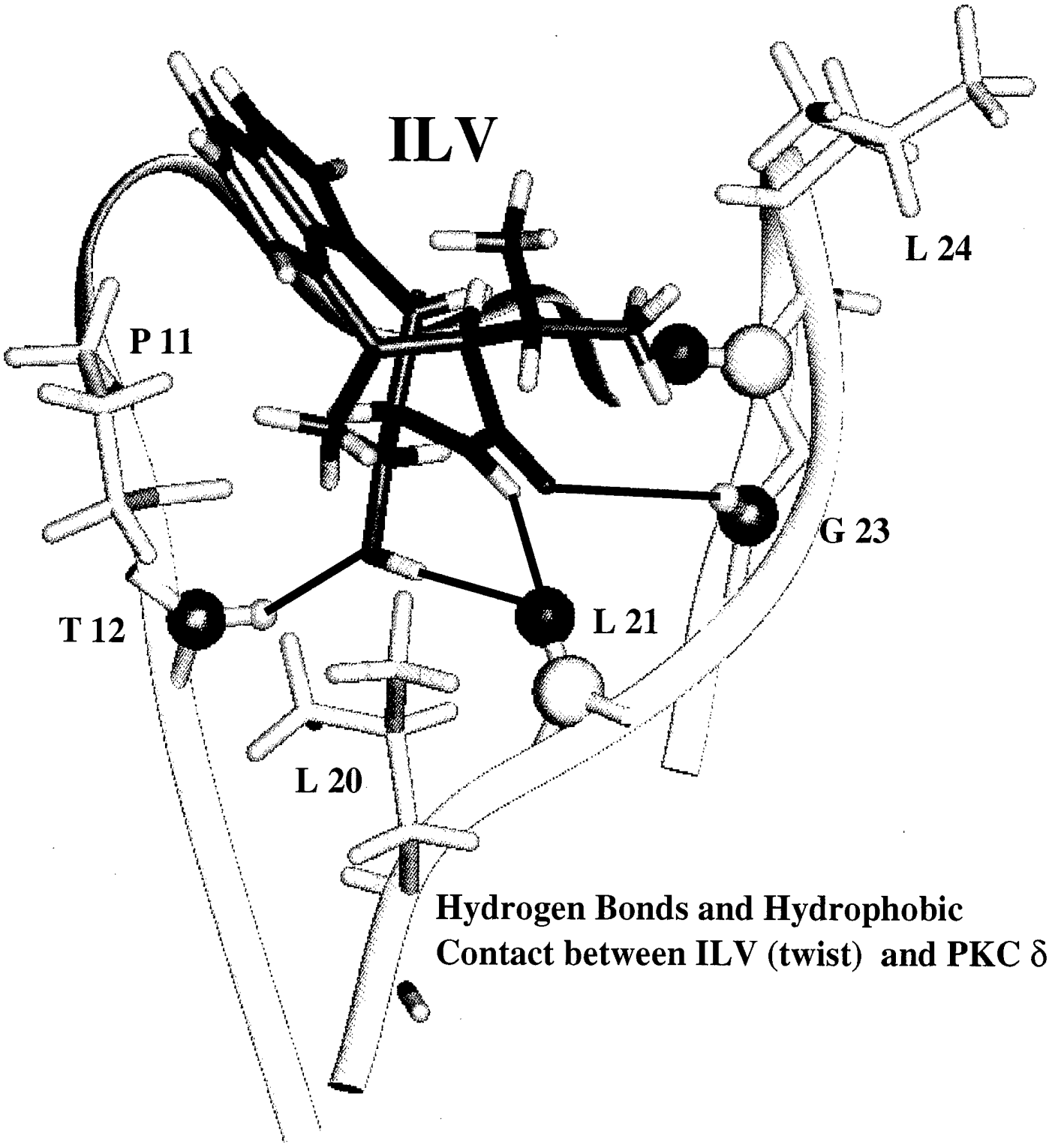
After he joined the GICCS in July, 1996, he has undertaken the task of designing isozyme selective PKC ligands, in collaboration Dr. Alan Kozikowski who has been worked on the chemistry of a novel class of PKC ligands, indolactam V (ILV) for the last few years. Using his molecular modeling expertise, he determined how ILV is being recognized by PKC, a problem that researchers in PKC field have tried to solve but failed in more than a decade. The interactions between PKC and ILV are shown in Figure 1. The understanding on the precise binding features of ILV to PKC has already led to the design and discovery of a novel PKC ligand with considerable isozyme selectivity. This kind of potent, highly selective PKC ligands will find use in a number of areas, including apoptosis, Alzheimer's disease and cancer. A paper has been written and submitted to Journal of the American Chemical Society (JACS).

He has joined force with Dr. Alan Kozikowski, in an effort to develop novel cognition enhancing agents based upon Huperzine A (HA), a potent naturally occurring reversible inhibitor of acetylcholinesterase (AChE) that has been used in China to treat Alzheimer's Disease patients. Molecular modeling has been employed to study the precise binding nature between HA and AChE. The predicted binding site of HA in AChE, as shown in Figure 2, is in excellent agreement with a recently determined X-ray structure of HA in complex with AChE. Through the molecular modeling studies of the binding site of HA in AChE, he identified a number of possible modifications in HA that could lead to analogues with improved activity. Molecular modeling studies have been used to study the binding of a superior AChE inhibitor, the C10-methyl HA analogue, to AChE. This analogue is 8 times more potent than HA and has great potential to be developed as a drug to treat AD patients. A paper concerning this study is in press in JACS.

He has established a number of other exciting collaborations after he joined the GICCS. He initiated a collaboration with Dr. Alan Faden's group, aimed at the discovery of novel and non-peptide inhibitors for apopain/CPP32, a key mediator of apoptosis, using the 3D-database pharmacophore searching technique he developed at NCI. He started a collaboration with Dr. Kenneth Johnson at University of Texas to discover cocaine antagonists as potential treatment for cocaine abuse.

Dr. Wang is expecting 1997 being a very productive year.

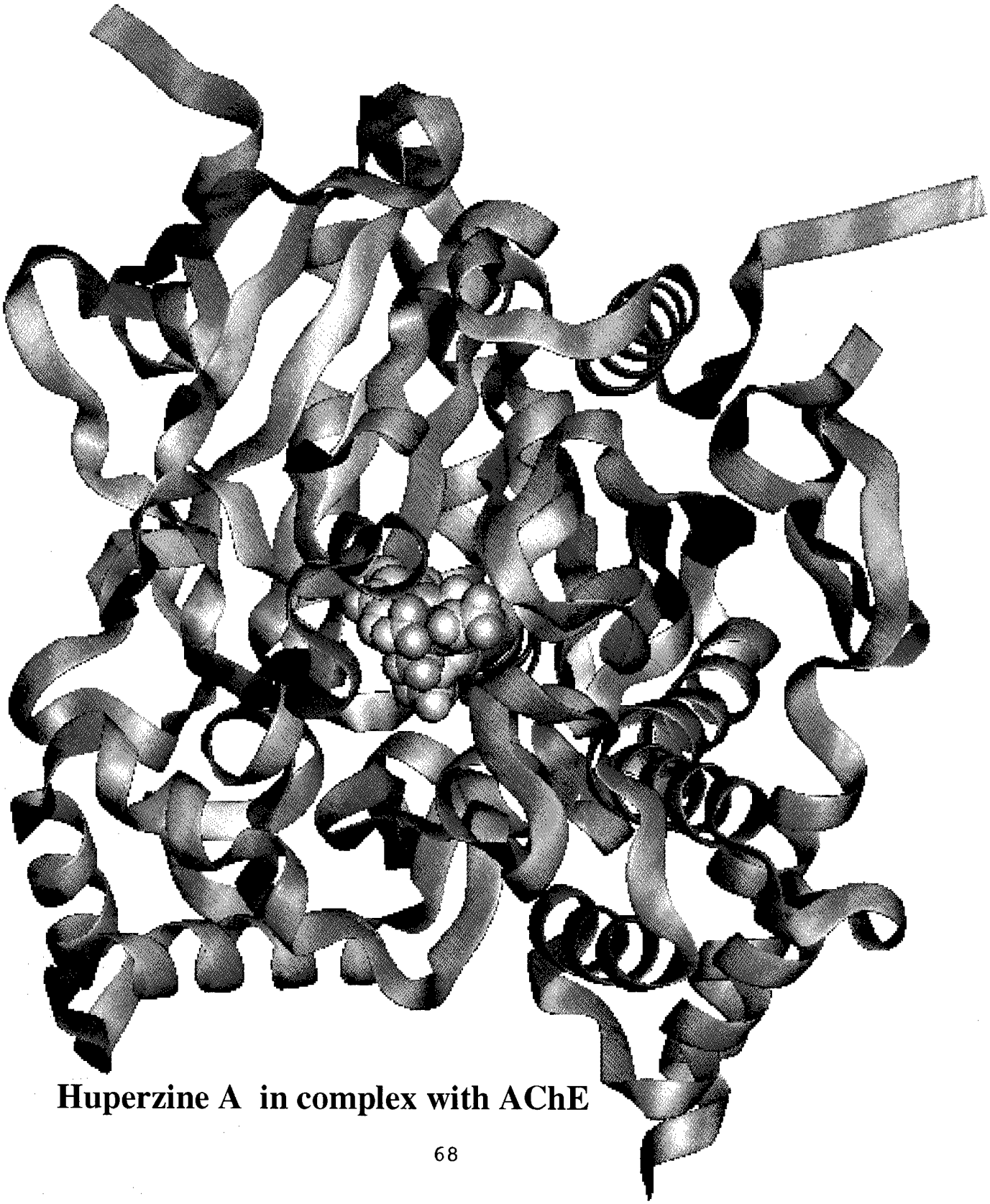
Figure 1. Molecular modeling studies of the binding between Indolactam V and protein kinase C.



Hydrogen Bonds and Hydrophobic Contact between ILV (twist) and PKC δ

Shaomeng Wang

Figure 2. Molecular modeling studies and structure–based design of AChE inhibitors based upon Huperzine A as potential drug for the treament of AD patients.



Huperzine A in complex with AChE

MOLECULAR NEUROBIOLOGY AND PLASTICITY: There are three faculty in this research area. Dr. Etcheberrigaray evaluates molecular mechanisms of Alzheimer's disease with particular reference to a novel potassium channel and a novel G- protein. Dr. Swope's research relates to the actions of novel receptor protein kinases. Dr. Faden's group evaluates the molecular and cellular correlates of secondary neuronal injury following trauma, including apoptosis.

RENÉ ETCHEBERRIGARAY, M.D.

Introduction

The principal aim of the research program is to study molecular alterations in Alzheimer's disease. The research focuses on ion channels (potassium channels, IP3 calcium release channel), second messengers (intracellular calcium, inositol cascades), and phosphorylating enzymes such as protein kinase C. These molecules (and associated cellular procesees) have been implicated in mechanisms of memory storage and also in cell toxicity events. Thus, their alterations may be of particular relevance in AD pathophysiology. Fibroblasts are the principal cellular model for these studies. Fibroblasts have been successfully used to study neurological disorders and they provide a system where molecular changes may be studied without interference of a more generalized pathological process such as the one that occurs in the AD brain . In addition, I have started projects to study the same or similar molecular changes in brain tissues from animal models and AD patients. To accomplish these goals, during the past year I devoted significant efforts to develop state -of- the- art laboratory facilites for cell culture, electrophysiology and calcium regulation studies. In addition, a carefull search , selection and training of postdoctoral fellows was conducted. A brief description of the main laboratory components and personnel to follow.

Laboratory

Patch clamp. Modern patch clamp setup includes Axon Instruments amplifiers and computer based data acquisition hardware. The latest software version (Axon Instruments) for data acquisition and analysis has been installed on a Pentium based computer. In addition to computer files, a PCM/VCR is used for data storage. Tektronix digital oscilloscope allows on-line monitoring of the signals. Experiments are performed with a Zeiss 135 inverted microscope. Patch clamp pipettes are driven with a Burleigh piezoelectric micromanipulator.

Calcium imaging. State-of- the-art Zeiss-Attofluor, Ratio Arch system has been installed coupled to the patch clamp microscope for intracellular calcium measurements. Imaging card, filter changers and UV lamps are controlled by Attofluor software installed on a customized Pentium based computer.

Lipid bilayers. Custom made lipid bilayer chambers (Micron Systems) are connected to amplifiers similar to those used in the patch clamp setup. Oscilloscope and a PCM/VCR storage unit are also installed.

Cell culture. Dedicated cell culture facility has been installed for handling human fibroblasts cell lines.

Cell dissociation. Cell dissociation units (custom made, Micron Systems) were installed to acutely dissociate differentiated neurons from mammalian brain.

Recruitment of Postdoctoral Fellows

The search for postdoctoral fellows was initiated in mid November, 1995 by advertising at the Society for Neuroscience Meeting and in the magazine, Science. From approximately 50 applicants, about 30% were initially interviewed by telephone. The most qualified individuals were personally interviewed resulting in the successful recruitment of Dr. Seetha Bhagavan (Ohio State University) in March 1996 and of Dr. Huafeng Wei (George Washington University) in June 1996.

Project 1: Fibroblasts Studies

Introduction

Recent research has identified consistent molecular alterations in fibroblasts from AD patients as compared to controls including young, age-matched, and individuals suffering from other neurological and psychiatric conditions. Briefly, we have identified a dysfunctional K^+ channel and an enhanced IP_3 mediated calcium release in fibroblasts from AD patients. In addition, the K^+ channel defect was induced by -amyloid treatment (10 nM) in otherwise normal fibroblasts. These studies have provided the bases for potential diagnostic methods using combined molecular changes as well as new venues on understanding the pathophysiology of AD. In addition to the above mentioned changes, alterations in protein kinase C have been found in fibroblasts and brains of AD patients. Studies have found reduced levels of PKC in brains and in fibroblasts of AD patients. Immunoblotting analyses revealed that only the isoform was significantly reduced. PKC may also play a role in APP processing since it contains phosphorylation sites and PKC activators influence the type of secreted APP products. It has been shown that PKC activation can increase the rate of the non-amyloidogenic processing in cellular models. Therefore, the general finding of reduced PKC amounts and/or activity in AD (brain and fibroblasts) is consistent with the apparently normal regulatory role of PKC favoring non-amyloidogenic processing of APP. My principal goal is to understand the potential involvement of protein kinase C in the context of the above mentioned K^+ channel alterations and to provide a pathophysiological important link between these alterations.

We have suggested that the induction of an upregulation of PKC may result in restoring the normal potassium channel function in AD fibroblasts. This upregulation could also prevent or revert the potassium channel defects induced by β-amyloid. Experimental confirmation of this hypothesis could provide evidence for a major and perhaps primary role for PKC in this disease. Defective PKC might lead to an increase in β-amyloid production, which in turn might affect potassium channels. We could also speculate that these chain of events may ultimately result in defective memory mechanisms and related clinical manifestations.

It has been previously established that the AD cell lines that do not have "functional" 113-pS TEA-sensitive K^+ channels, do not exhibit significant calcium elevations in response to TEA- induced depolarization. Calcium imaging techniques will be used to assess calcium responses in previously documented "unresponsive" cells treated with PKC activators. This constitutes an additional and complementary assessment of K^+ channel function that allows the measurement of channels in the entire plasma membrane and in a large number of cells simultaneously.

Methods and Results

The cell culture procedures are as published, except that cells were seeded in 2.5 mm glass cover slips and not in plastic dishes. Calcium measurements were as follows: culture medium was removed and cells were washed at least three times with BSS (in mM: NaCl 140, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 1.5, HEPES 10, Glucose 5, pH= 7.4). The fluorescent probe was loaded by incubating the cells in 1 &M (in BSS) fura 2-AM (Molecular Probes) for 60 min at room temperature. After loading, cells were washed thoroughly with BSS. After washes, 1 ml of fresh solution was added for intracellular Ca^{2+} baseline measurements. TEA (Sigma) challenge was performed by adding to the dish 3 ml of TEA-modified BSS (TEA-MBSS) solution (in mM: TEA 133.3, NaCl 6.7, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 1.5, HEPES 10, Glucose 5, pH= 7.4). This solution was adjusted in order to introduce no osmolariity changes. The 334 /380 nm ratios were acquired at a rate of 1/sec. for about 300 sec, with a Zeiss-Attofluor system. The objective lens was a 40x Zeiss Fluar (oil immersion). A group of cells were treated with the PKC activator benzolactam (50 nM in DMSO) for 1 min prior to the TEA challenge. At least seven petri dishes of cells of a given cell line were used for each experimental condition. A second group of cells were treated with 50 nM of the PKC activator 12-13- phorbol dibutyrate (PDBu) for 1, 15 and 45 min before TEA application. The number of cells measured per cell line and condition was > 50. In a given cell, an elevation of at least 100% from base line was considered a "response" . Experiments for each cell line were repeated on at least one separate occasion (minimum one week interval).

Baseline TEA responses (measured as % of cells responding) in four cell lines from AD patients were as a group below 10%. Three out of four cell lines had, as expected, minimal responses. One of the

cell lines had a somewhat higher than expected % of responding cells(16%). The novel, isozyme selective PKC activator ( ) benzolactam was tested in three cell lines. One min. incubation was sufficient to cause a significant increase in the % of cell responding to TEA. The well known, non-selective PKC activator PDBu was also used. After PDBu incubation for 45 min, TEA application resulted in a significant increase in the % of cells responding to the challenge in only one of the tested cell lines. It was without effect in two and had a modest effect in one. Shorter incubation times were without effect in all cell lines. The combined control group (DMSO, 4 phorbol, and untreated) had responses significantly lower than benzolactam-treated cells but did not differ from the PDBu-treated group. Benzolactam alone, independent of TEA, was capable of inducing immediate calcium elevations in about 18 % of the cells studied. Further quantification would be required to assess the nature and significance of this finding. Fig. 1 depicts a typical fluorescence image of the cells. The two types of response profiles are summarized in Fig. 2 and the results are graphed in Fig. 3. Future experiments will include the use of additional isozyme-selective PKC activators and dose/incubation dependence studies. Ion channels will be also directly evaluated with the patch clamp technique.

Project 2: Mammalian Brain Studies

Introduction

In spite of the advances in identifying relevant molecular changes in peripheral tissues, it remains important to determine whether or not similar changes take place in the central nervous system, the primary site of AD pathology. Nevertheless, studying ion channels (and IP3 receptors) in human brain cells, specially from post-mortem material, constitutes a major technical challenge. Acute cell dissociation, although theoretically possible, may not give enough viable cells to make it a practical approach. There is, however, an alternative procedure that has the potential to circumvent most of these obstacles to study ion channels from brains of AD patients. The artificial lipid bilayer technique allows for the study of ion channels in a cell free system, from a variety of protein sources including purified protein channel, channels from intracellular and extracellular vesicles and or membranes, and from synaptosomes. In addition, viable synaptosomes have been successfully obtained from human brain autopsy material. Thus, synaptosomal membranes from AD brains can be incorporated in lipid bilayers and ion channels studied. As a preliminary and preparatory step, we have been preparing synaptosomal membranes from experimental animals (gerbils) and electrophysiologically and pharmacologically characterizing K^+ channels incorporated in lipid bilayers.

Methods and Results

Synaptosomes were prepared following standard procedures involving homogenization, and sucrose gradient ultracentrifugation. Synaptosomes were broken by freeze and thawing just before the experiments. Membranes were added to the one of the chambers (cis) and fusion was promoted by an osmotic gradient and magnetic steering of the chambers. The solution in the cis and trans chambers are K acetate at 50 mM and 500 mM concentrations, respectively. The chambers (separated by the artificial bilayer made of PS/POPE) are connected to Axon amplifiers to monitor and measure ion channels when incorporation occurs.

Potassium channels from synaptosomes have been successfully and routinely incorporated into the lipid bilayers. Four major types of K channels have been identified and partially characterized. Type I K^+ channel has the largest conductance of 339Å3 pS. Calcium is required to observe this channel's activity. Membrane depolarization increases its probability of opening. Type II K^+ channel has conductance of 327Å7 pS. Its activity does not depend on the presence of Ca^{2+} and its probability of opening is decreased by membrane depolarization. This K^+ channel is blocked by $CsCl_2$ (50 mM) but not by charybdotoxin (100 nM) or -dendrotoxin (50 nM). In contrast to type I and type II K^+ channels, type III K^+ channel (258Å5 pS), is characterized by flickering pattern and its probability of opening is not voltage-dependent. Type IV K^+ channel possesses a conductance of 150Å4 pS. Like type III K^+ channel, it has a flickering pattern but membrane depolarization increases its opening probability. The probability of opening of this K^+ channel is dependent on the presence of Ca^{2+}, and can be inhibited by $BrCl_2$ (10 mM) but not by tetraethylammonium (TEA, 100 mM). Representative traces of one of this channels, its current(I)/voltage (V) relationship and

Human Fibroblasts

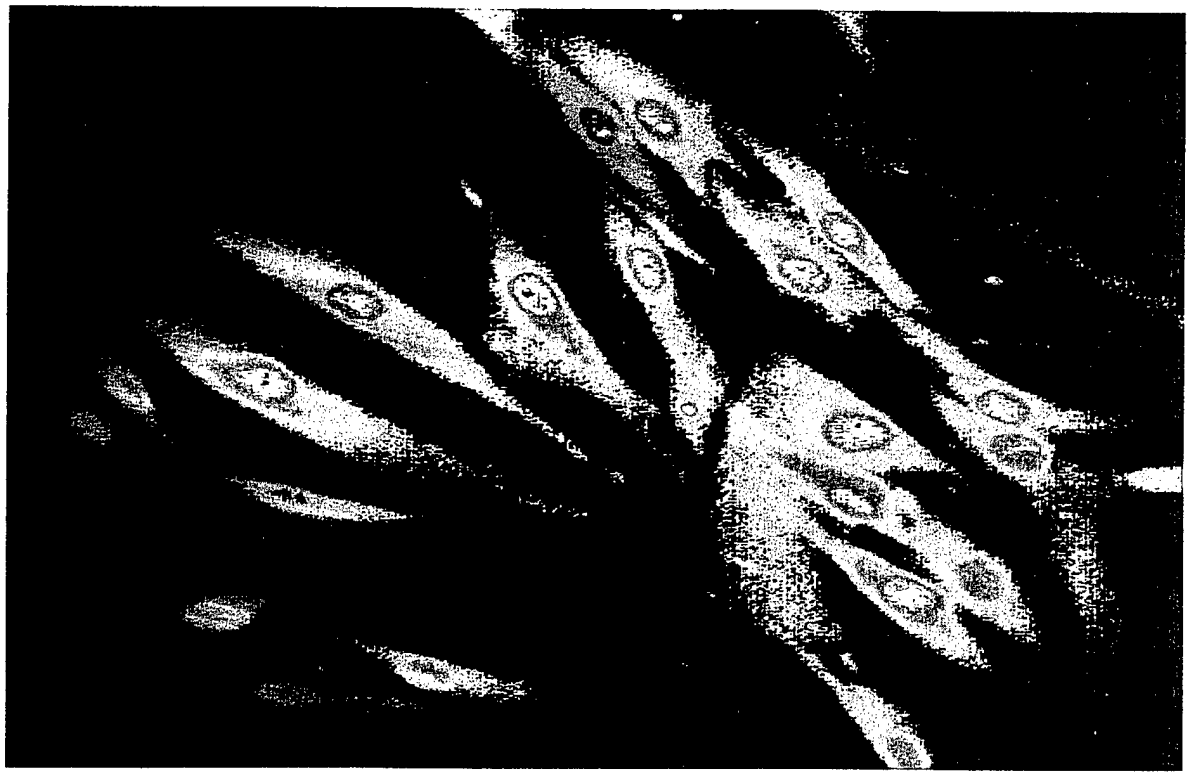


Figure 1

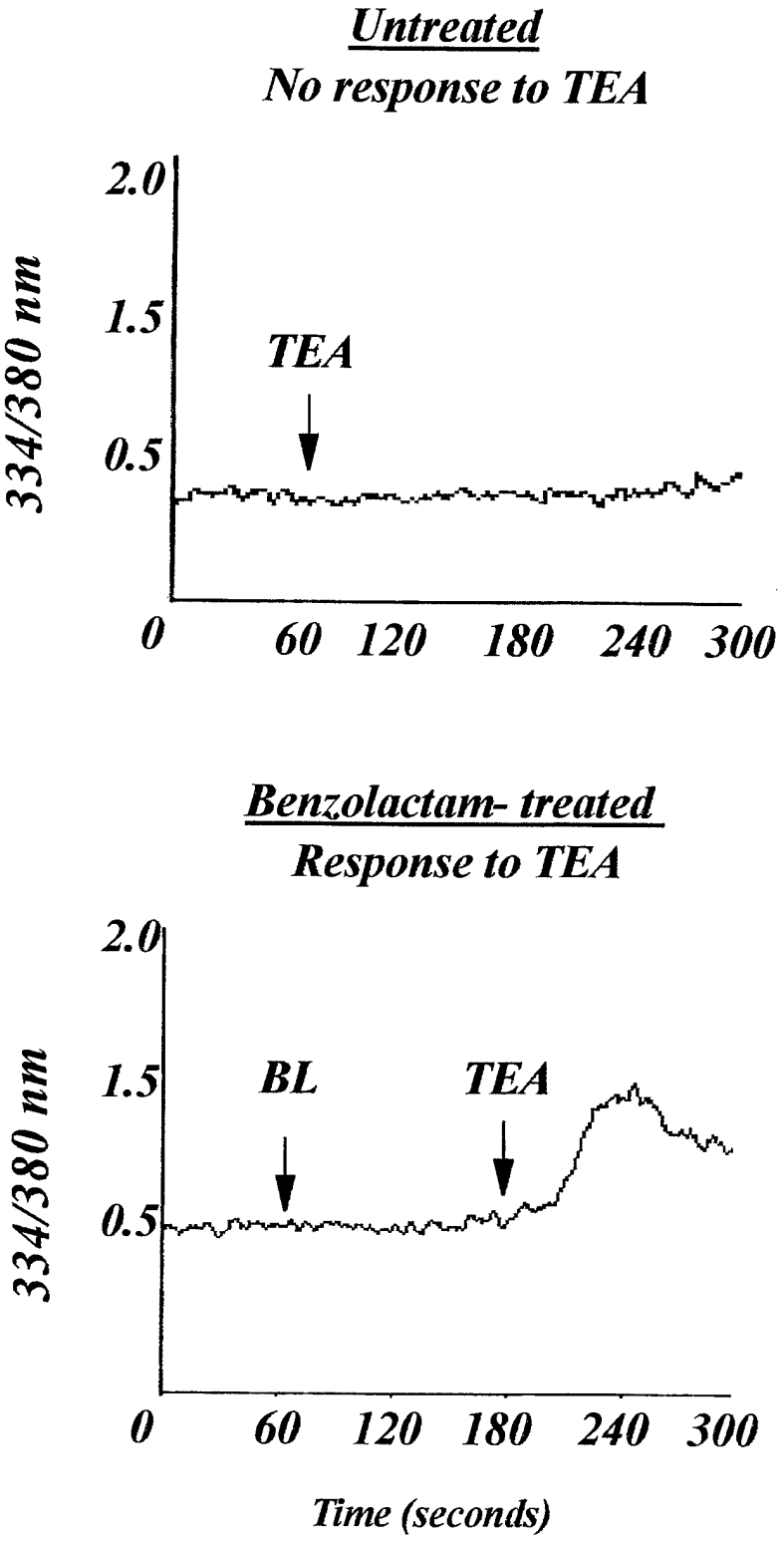

Untreated
No response to TEA
2.0
1.5
0.5
334/380 nm
TEA
0
60
120
180
240
300
Benzolactam- treated
Response to TEA
2.0
1.5
0.5
334/380 nm
BL
TEA
0
60
120
180
240
300
Time (seconds)

Figure 2

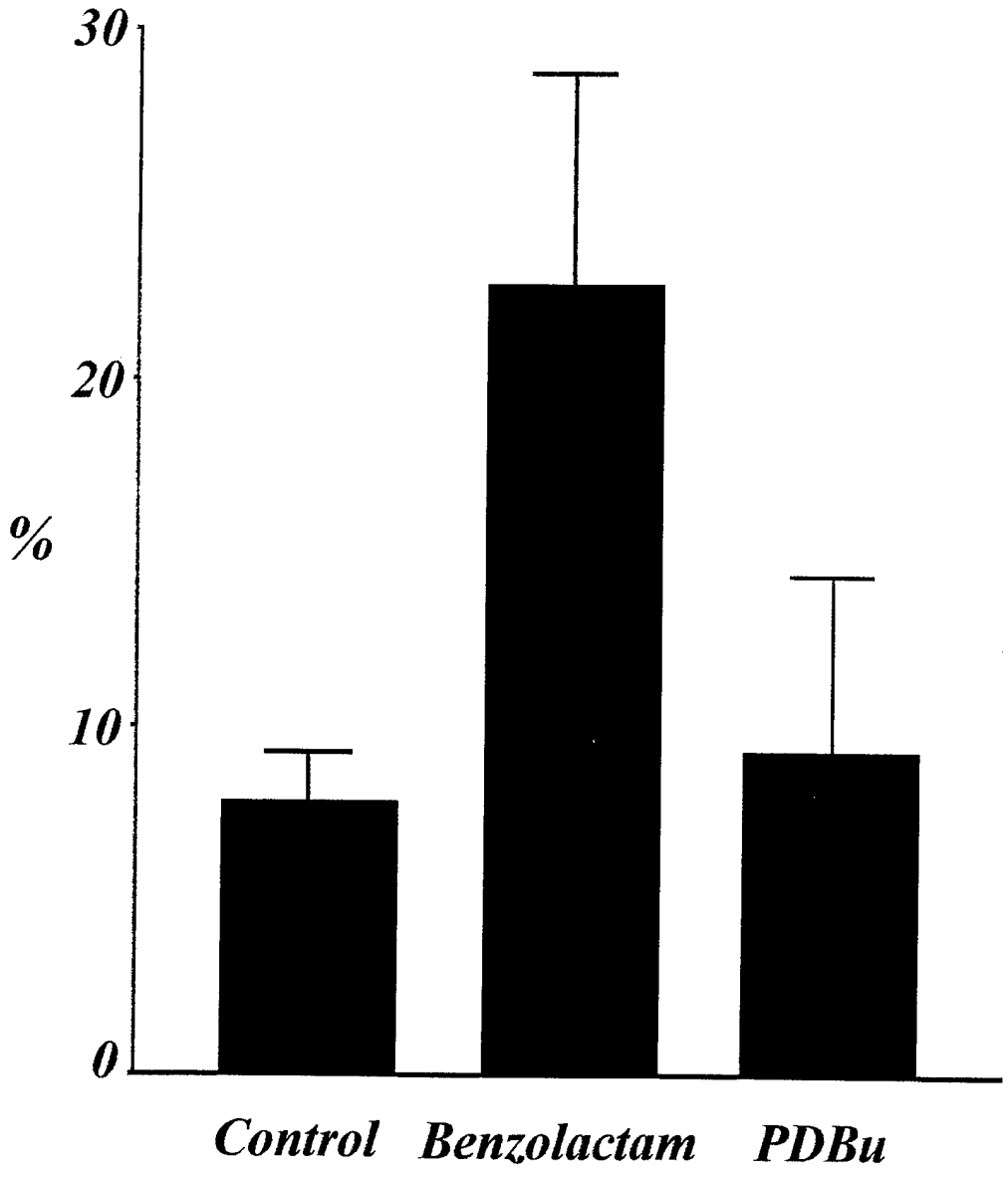
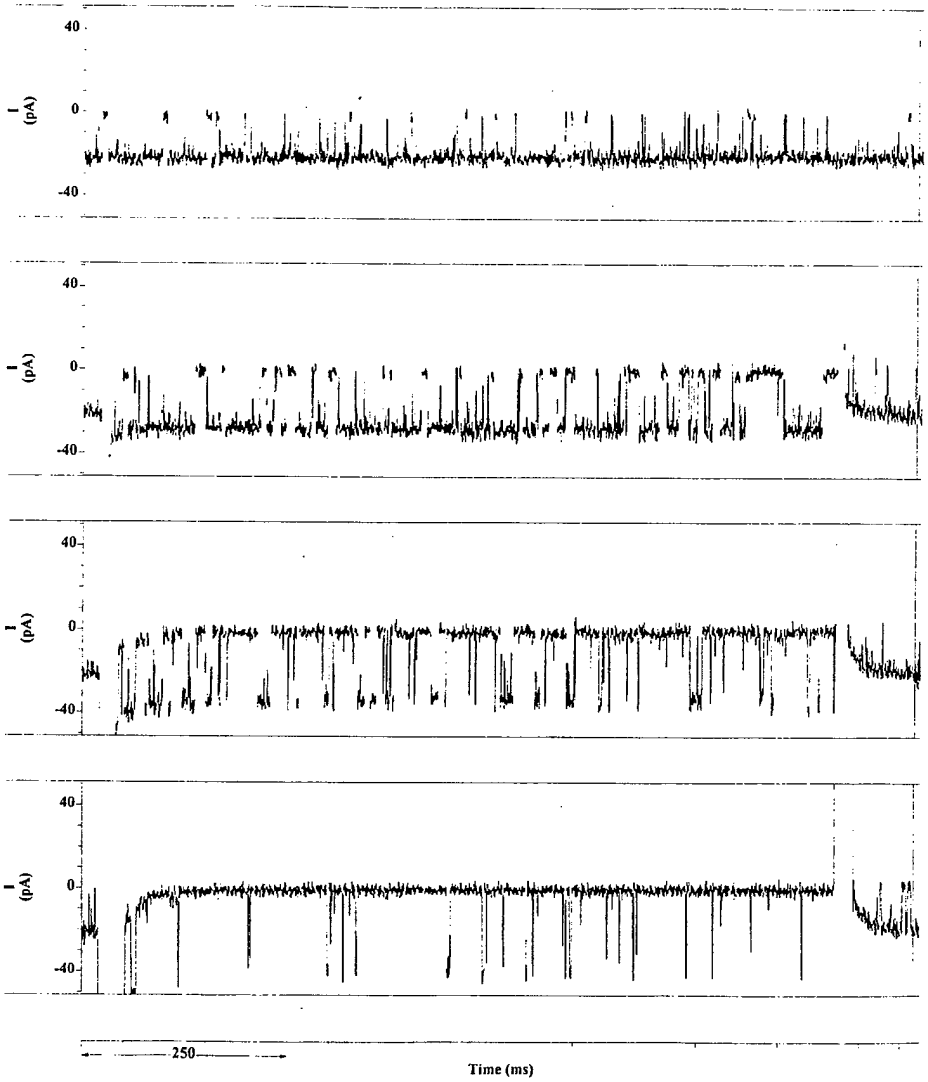
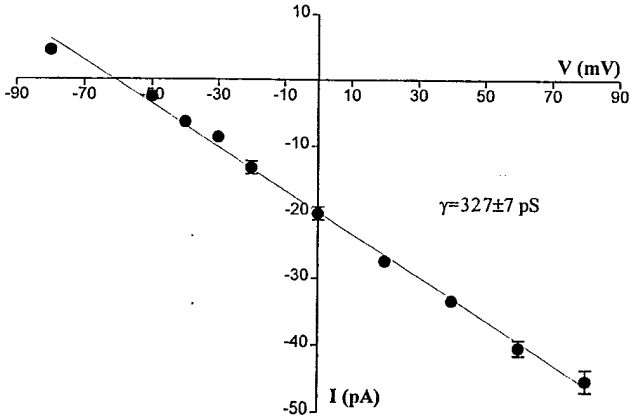


Figure 3

GK-II IV data, 0 to 60 mV by 20 mV (top to bottom)





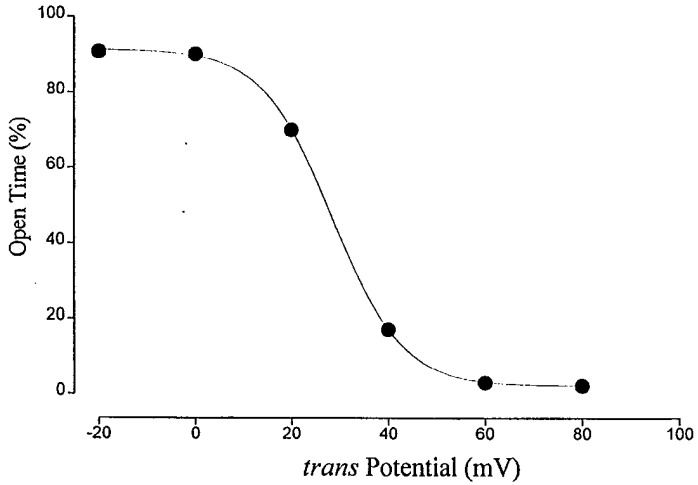


Figure 4
75

kinetic analysis are depicted in Fig. 4. This study constitutes the baseline work for characterizing K^+ channels from AD patients' and control's brains.

Conclusions and Future Directions

The successfull development of all major components of the laboratory has been completed. As described in detail above, two major research effors are in progress. Significant amount of data has already been collected. Preliminary results strongly suggests that PKC might play a major role in AD. We have also completed the preliminary work that will allow the study of ion channels in AD brains. Completion or significant advancement of these two projects constitute the main goal for the next year. Further assement of PKC function in AD will include immunoblott analyses. Collaborative research efforts on related subjects such as brain injury and ischemia will also be developed.

ALAN I. FADEN, M.D.

Dr. Faden focuses on the chemical pathobiology of secondary tissue damage following CNS trauma and utilizes multidisciplinary approaches including molecular and cellular neurobiology, neuropharmacology, behavioral evaluation (including cognitive) and magnetic resonance techniques. Four parallel but independent lines of investigation are supported in part by the Institute. These are detailed below:

Project 1: Role of Ced-3 Relate Cysteine Proteases (CRCP) in Neuronal Apoptosis

Project 1A: Role of CRCP in Apoptosis of Cerebellar Granule Cells

Introduction

Injury to the central nervous system can cause both immediate and delayed death of neurons. A portion of delayed neuronal death has been attributed to apoptosis, a form of programmed cell death characterized by nuclear condensation, chromatin margination, plasma membrane blebbing, and cytoplasmic fragmentation in the absence of an inflammatory response (Rink, et al, 1995 and Bredesen, 1995). One of the stimuli leading to neuronal apoptosis after CNS injury may involve a withdrawal of trophic support from either growth factors or transmitter-mediated synaptic activity. As such, cultures of cerebellar granule cells can serve as a model of apoptosis induced by the deprivation of trophic support from serum and/or potassium ions (Atabay, et al, 1996 and D'Mello, et al, 1993).

A group of proteases known as the Interleukin 1β-Converting Enzyme (ICE) family of cysteine proteases has been implicated as potential mediators of the apoptotic cascade. These proteases are the mammalian homologues of the C. elegans ced-3 pro-apoptotic gene product and can be divided into two classes of enzymes: the ICE-like proteases and the CPP32-like proteases (Martin and Green, 1995). The CPP32-like proteases, which include CPP32, ICH-1, MCH-1, and a growing list of others, can cleave several functionally important proteins such as poly (ADP-ribosyl) polymerase (PARP), an enzyme involved in DNA repair, and lamin B, a cytoskeletal element associated with the nuclear membrane. The cleavage of these two substrates may contribute to the appearance of DNA fragmentation and to the changes in nuclear morphology during apoptosis, respectively. Both of these proteins contain the sequence Asp-Glu-Val-Asp (DEVD), which is recognized specifically by CPP32-like proteases and cleaved adjacent to the C-terminal aspartate (Tewari, et al, 1995; Lazebnik, 1995; and Nicholson, et al, 1995).

While PARP, lamin B, and other substrates have been shown to be cleaved by CPP32-like proteases in vitro, the importance of this activity in neuronal apoptosis in unclear. Consequently, the activity of CPP32-like proteases in the apoptotic death of cerebellar granule cells was investigated, and in addition, the proteolytic activity of CPP32-like proteases was correlated with the transcription of CPP32 itself.

Methods and Results

Preparation of Cerebellar Granule Cells

Cerebellar granule cells were prepared from 8-day-old rat pups. Briefly, cerebella were dissected and sectioned on a tissue chopper, followed by trypsinization and trituration. Approximately 1.25 x 10^6 cells/ml were seeded onto 60mm poly-L-lysine coated dishes in Basal Medium Eagle (BME, Gibco) containing 10% fetal bovine serum and 25mM KCl. At 8 days in vitro, cells were washed with BME, and the medium was replaced with 25mM KCl conditioned medium (Ctrl), 25mM KCl/serum-free medium (serum deprivation), 5mM KCl conditioned medium (KCl deprivation), or 5mM KCl/serum-free medium (serum/KCl deprivation). Incubations were terminated after 1, 4, or 12 hours.

Terminal deoxynucleotidyl transferase-mediated dUTP-biotin Nick End Labeling (TUNEL)

The extent of apoptosis was quantified by Terminal deoxynucleotidyl transferase-mediated dUTP-biotin Nick End Labeling (TUNEL) of DNA fragments (Gavrieli, et al, 1992). Cells were fixed in methanol for 5min and dried. A section of cells was then rehydrated with PBS and permeabilized in 0.1% saponin. DNA fragments were tailed with biotinylated nucleotide and detected with streptavidin/horse radish peroxidase-catalyzed precipitation of TACS® Blue Label (Trevigen, Inc.). Finally, cells were counterstained with Nuclear Fast Red.

Data are expressed as the mean ratio of apoptotic cells to non-apoptotic cells per high powered field (n=3). As shown in figure 1, serum deprivation appears to have no effect on apoptosis after 12 hours. Potassium deprivation produces a modest increase in apoptosis and combined serum/potassium deprivation produces a significantly greater increase.

DNA Fragmentation Analysis

Total genomic DNA was extracted by addition of 7M guanidine hydrochloride followed by adsorption to Wizard Minipreps DNA Purification Resin (Promega). The resin-DNA suspension was passed through a filtration column and DNA was eluted with water. Samples of approximately 500ng DNA were electrophoresed in 1.2% agarose and visualized by ethidium bromide fluorescence. The prominence of the DNA ladders appears to correlate with the TUNEL data.

CPP32-like Protease Activity

Total cellular protein was extracted in a CHAPS-based buffer. For each sample, a 20μg protein aliquot was incubated at room temperature with 20mM Ac-DEVD-aminomethylcoumarin (AMC), a specific fluorogenic substrate for CPP32-like proteases. Cleavage activity was measured by fluorometry of the initial velocity of free AMC accumulation at 360nm excitation and 460nm emission.

The data are expressed as the percent of activity in control-treated cells (n=4). Like the TUNEL and DNA fragmentation analysis data, CPP32-like activity appears negligible in serum-deprived cultures, moderately increased in potassium-deprived cultures, and significantly increased in combined serum/potassium-deprived cultures.

CPP32 mRNA Expression

Total RNA was extracted in guanidine isothiocyanate/acidic phenol/chloroform and precipitated in isopropanol. Following digestion of residual DNA, 1.25 μg total RNA per sample was reverse transcribed, and 125ng were amplified by polymerase chain reaction using primers specific to CPP32 or glyceraldehyde-3-phosphate dehydrogenase (GAPDH). One third of the total reaction volume from each sample was electrophoresed through 2% agarose and visualized by ethidium bromide fluorescence. The mean optical density of each band was determined by densitometry of the digitized image.

The data are expressed as the ratio of the mean optical density of the CPP32 product to the GAPDH product with standardization to control–treated cells. While there is little change in CPP32 mRNA expression in serum-deprived cells, the most significant increase appears in potassium-deprived cells.

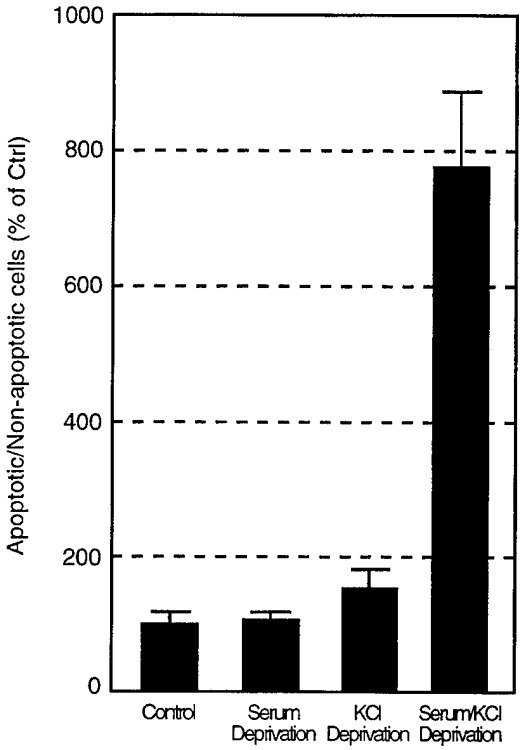


Fig. 1. TUNEL assay 12 hours after treatment. Serum deprivation potentiates the apoptotic cell death induced by KCl deprivation.

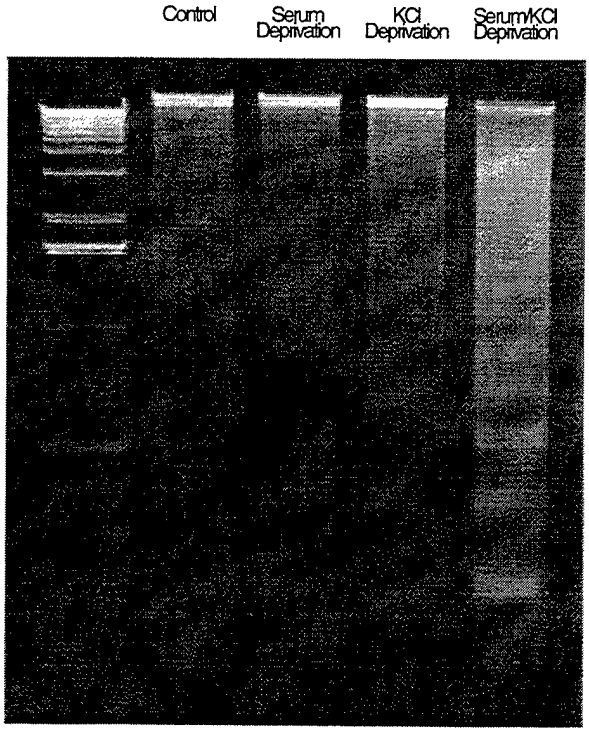


Fig. 2. DNA fragmentation analysis 12 hours after treatment. The degree of DNA laddering appears to correlate with the results of the TUNEL assay.

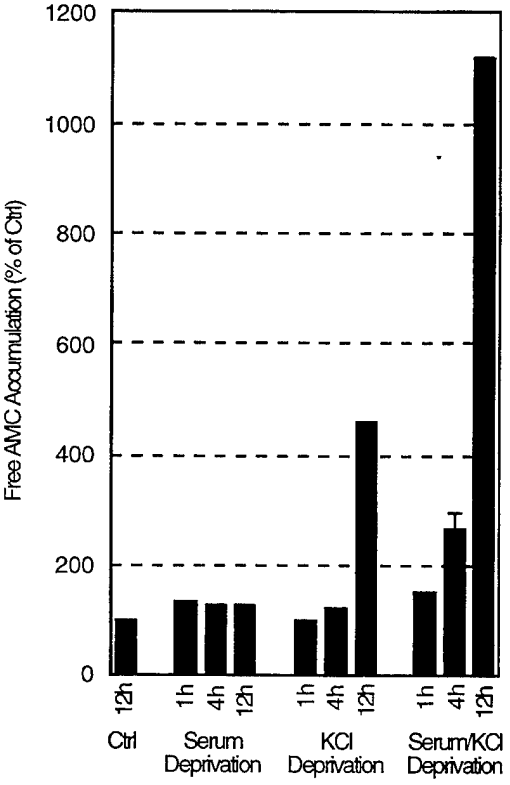


Fig. 3. CPP32-like protease activity. Serum deprivation potentiates activity induced by KCl deprivation.

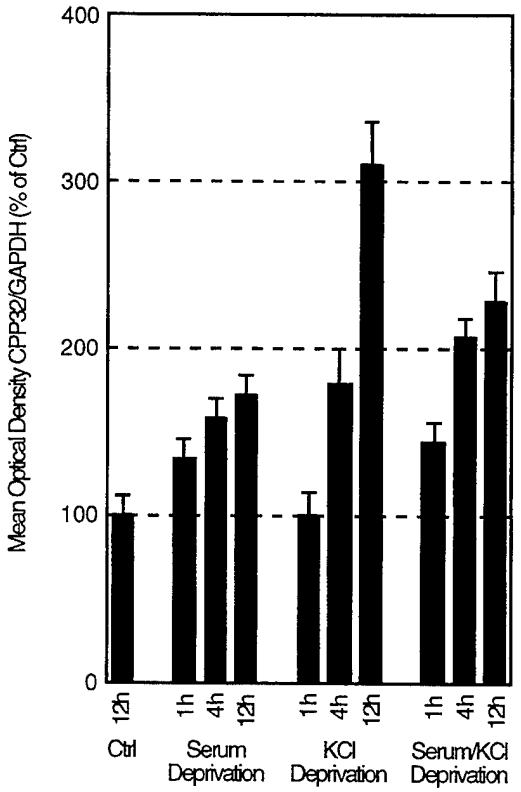


Fig. 4. CPP32 mRNA expression. After 12 hours the concentration of CPP32 mRNA is greatest in the KCl deprived condition.

While serum deprivation appears to have no effect on the extent of apoptosis (figs. 1&2), CPP32-like protease activity (fig. 3), or CPP32 mRNA expression (fig. 4) at the timepoints examined, it does potentiate the increases in apoptotic death and CPP32-like activity after potassium deprivation. Cysteine protease activity is dependent in part on the cleavage of the proenzyme precursor; however, activity may also be dependent on transcriptional regulation. To investigate this question, RT-PCR was used to quantify the changes in CPP32 mRNA expression. As shown in figure 4, CPP32 mRNA expression increased significantly after potassium deprivation but only modestly after combined serum/potassium deprivation. Therefore, the increased activity of CPP32-like proteases in the latter condition may be due to the increased transcriptional rate of another protease in the CPP32 class. Alternatively, activity in this condition may be dependent on translational rate or proteolytic activation from the proenzyme form.

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Project 1B: Role of CRCP in Apoptosis Induced by Traumatic Brain Injury

Introduction

Neuronal cell loss following CNS injury appears to result from apoptosis as well as necrosis. Although apoptosis has been demonstrated in brain after experimental traumatic brain injury (TBI), a critical role for apoptosis in posttraumatic tissue damage and associated neurological disability has yet to be established. Moreover, little is known about the molecular mechanisms involved in apoptosis after CNS injury. Preliminary studies from our laboratory suggest that certain cysteine proteases that share sequence homology to the Ced-3-nematode "death gene", particularly ICE and CPP32, may play an important role in posttraumatic apoptosis in rats subjected to fluid percussion-induced TBI.

Methods and Results

We analyzed DNA fragmentation in rat brain as a function of time after traumatic injury and examined both the transcription and catalytic activation of Ced-3-related cysteine proteases. There were time-dependent increases in DNA fragmentation in samples from cortex and hippocampus ipsilateral to the trauma, but not in samples contralateral to the trauma; changes were observed as early as 4 h after trauma, with marked DNA laddering observed at 3 days (Fig. 5). Consistent with DNA fragmentation was the presence of ISEL positive-staining cells in sites ipsilateral to the injury. Levels of mRNA expression were measured using RT-PCR: CPP32 mRNA was markedly increased in ipsilateral cortex and hippocampus following trauma, whereas ICE mRNA increases were more modest (Fig. 6)

To demonstrate possible modulation of apoptototic DNA cleavage activity in brain tissues by traumatic injury, we adopted a recently developed model of reconstitution of apoptosis *in vitro*. Nuclei isolated from rat liver were incubated for various periods with the cytosolic extracts isolated from injured cortex 3 days after TBI and DNA was analyzed by agarose gel electrophoresis. Prominent fragmentation into a ladder of about 180 bp was observed within 60 min; the level of DNA fragmentation and isolated nuclei was dose-dependent. The degree of DNA fragmentation induced *in vitro* by cytosolic extracts from traumatized brain was consistent with the *in vivo* expression of apoptosis after trauma. To further analyze the potential role of CPP32-like proteases in trauma-induced apoptosis, we examined the effect of their inhibition by the tetrapeptide aldehyde Ac-DEVD-CHO. In the *in vitro* apoptosis model, DNA laddering was markedly reduced by tetrapeptide treatment (Fig. 7).

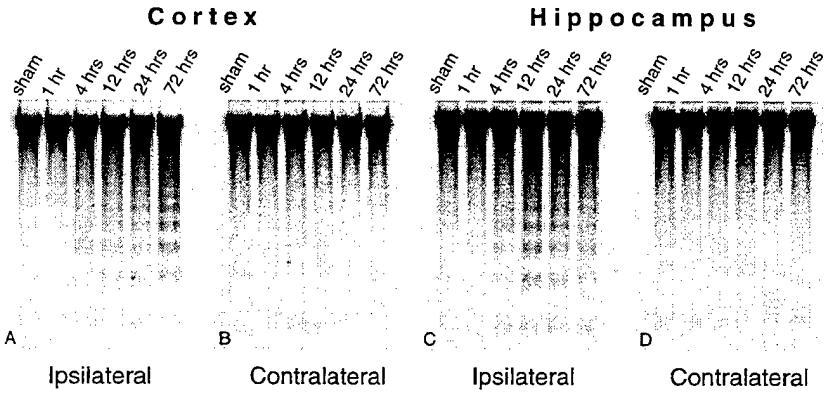

Figure 5: Agarose gel-electrophoretic analysis of genomic DNA isolated from traumatized (2 atm) and sham-operated rat brain tissues. Total DNA from ipsilateral (A) and contralateral (B) cortex, and ipsilateral (C) and contralateral (D) hippocampus was isolated at indicated time after TBI. Ten μg of DNA from each sample was end-labeled with [^{32}P] dATP and analyzed in 1.5% agarose gel. Dried gel was exposed to X-Ray film for 30 min.

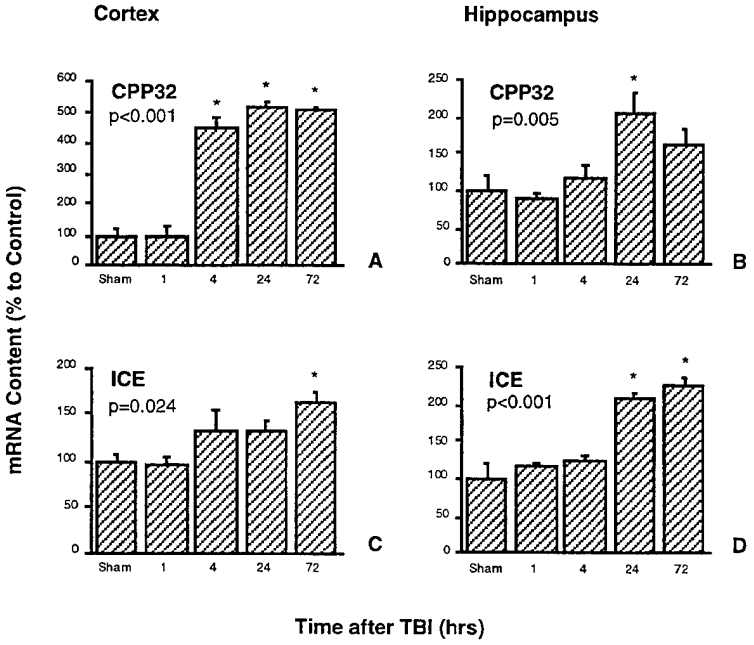

Figure 6: The time course of ICE (C & D) and CPP32 (A & B) mRNA expression in rat cortex (A & C) and hippocampus (B & D) after TBI (2 atm). Levels of mRNA are expressed in arbitrary units as the proportion of individual RT-PCR product mean optical density to GAPDH RT-PCR product optical density of the same RNA sample (n=3).

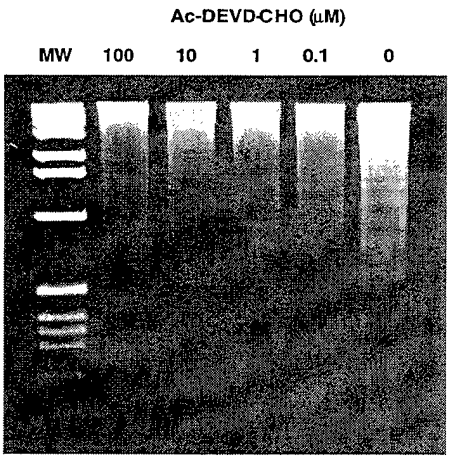

Figure 7: Effect of tetrapeptide aldehyde Ac-DEVD-CHO on *in vitro* apoptosis. The rat liver nuclei were premixed with Ac-DEVD-CHO or the reaction buffer to obtain the indicated final concentrations of tetrapeptide in a final volume of 60 μl. Cytosolic extract from injured cortex at 3 days after TBI was added to each sample at a final concentration of 6 mg/ml. Mixtures were incubated at 37° C for 30 min. and DNA was analyzed by agarose gel electrophoresis.

Conclusions

Taken together, our observations strongly suggest that (1) apoptosis occurs after lateral fluid percussion-induced TBI in ipsilateral cortex and hippocampus; (2) Ced-3-related cysteine protease genes, known to contribute to apoptosis in other models, appear to be transciptionally regulated posttrauma; (3) induction of Ced-3-related cysteine proteases may play a significant role in the initiation of apoptosis in neuronal cells after trauma.

Future Directions

We will examine the effects of tetrapeptide inhibitors of ICE and CPP32, as well as antisense oligodeoxynucleotides to these enzymes, on outcome (behavioral and histological) after TBI in rats.

Project 2 Traumatic Brain Injury and the Development of Alzheimer Disease

Introduction

Epidemiological data from humans indicates that traumatic brain injury (TBI) is a significant risk factor for sporadic Alzheimer disease (AD) (Mortimer et al, 1991). Recently, increases in the levels of amyloid precursor protein (APP), which liberates beta-amyloid when cleaved, have been observed in the rat CNS after fluid-percussion TBI (Pierce et al, 1996). Cognitive dysfunction, as assayed by the water maze test, has been noted by our laboratory and others to occur after fluid-percussion TBI in rats. We hypothesize that fluid-percussion TBI in rats will induce changes in the CNS which resemble those described in AD. Several 'markers' of AD have been described, in addition to progressive cognitive dysfunction. These markers include 1) decreases in calexcitan, a G-protein involved in learning and memory, 2) dysfunction of intracellular calcium regulation by inositol tri-phosphate (IP_3), and 3) changes in potassium channels. Calexcitan has been noted to be absent in fibroblasts and post-mortem brain tissue obtained from AD patients; and a decrease in the level of calexcitan appears to be predictive of AD. In

addition, the levels of calexcitan decrease in control fibroblasts incubated with beta-amyloid, a primary component of the pathological AD lesion (Kim et al, 1995). Agents which increase the levels of IP_3 induce a greater increase in intracellular calcium in AD fibroblasts than in controls (Ito et al, 1994). AD fibroblasts and olfactory neuroblasts exhibit a functional loss of a large-conductance TEA-sensitive potassium channel and a decreased sensitivity to TEA-induced calcium influx; beta-amyloid has been shown to induce similar changes in control fibroblasts (Etcheberrigaray et al, 1993; Etcheberrigaray et al, 1994). Furthermore, acute application of beta-amyloid in vitro blocks the fast-inactivating potassium current in early postnatal rat hippocampal neurons (Good et al, 1996), and ICV injections of beta-amyloid alter the TEA-sensitivity and profile of potassium channels in acutely dissociated hippocampal neurons from adult rats (Payne et al, 1995). We predict that after fluid-percussion TBI in rats 1) the CNS levels of calexcitan will be reduced, 2) dissociated hippocampal neurons will exhibit an increased sensitivity to agents which increase IP_3 levels, and 3) changes in the CNS potassium profile will occur.

We have designed primers for reverse-transcription polymerase chain reaction (RT-PCR) based upon the APP sequence, and we are currently investigating their specificity for APP mRNA. These primers will be used to analyze the levels of APP mRNA in different regions of the rat CNS after fluid-percussion TBI. To make it possible to examine changes in potassium channels and intracellular calcium, we have modified two techniques for use with adult rat CNS tissue. In order to isolate single neurons for patch-clamp and calcium imaging experiments, adult rat hippocampi are isolated, sliced, and then partially trypsinized. Individual cells are obtained by trituration through fire-polished Pasteur pipettes. With this technique, potassium channel recordings have been obtained from naive adult rat hippocampal neurons. Three potassium channel subtypes appear to be present in patches obtained from these cells Currently, these channels are being characterized for use as a comparison against those patches which will be obtained from traumatized animals. Preliminary studies are being carried out to determine the conditions necessary for calcium imaging of acutely dissociated hippocampal neurons. A second approach to the study of potassium channels involves artificial lipid bilayers. Adult rat hippocampi are centrifuged through sucrose gradients to obtain synaptosomal preparations, which contain channels embedded in membrane vesicles. These preparations are then allowed to fuse with a lipid bilayer separating an electrochemical gradient. We have obtained recordings of potassium channels from single adult rat hippocampi, and are currently characterizing these channels. Synaptosomes will be prepared from traumatized animals for study utilizing lipid bilayer recordings. Preliminary studies of the levels of calexcitan in naive and traumatized rats have been performed by Western blot analysis. Based upon the preliminary results, these studies are being replicated and extended with a more specific antibody against calexcitan.

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Project 3: Cognitive and Motor Consequences of Traumatic Brain Injury (TBI) Produced by Controlled Cortical Impact (CCI) in the Mouse

Introduction

Cognitive, motor and histopathological deficits commonly follow moderate to severe head injury in humans (Levin, 1996). Various animal models have been developed in order to gain a better understanding of the underlying mechanisms that contribute to these deficits. Such models have ranged from acceleration injuries in the primate to nonpenetrating focal deformation of the cortex in the rodent. Fluid-percussion injury to rat brain has been widely utilized to study both pathophysiological mechanisms and effects of pharmacolgical intervention (McIntosh et al, 1989, Faden, 1993). More recently, researchers have begun to develop mouse head injury models in order to examine the role of specific gene products in secondary injury (Smith et al, 1995). We have completed development and characterization of a controlled, direct mechanical injury model in mice to help us better address molecular and cellular mechanism of posttraumatic neuronal injury.

Methods and Results

The CCI injury device employed in these studies was designed and manufactured at GICCS and utilized a pneumatically-driven, microprocessor-controlled impounding tip. Cortical deformation was maintained constant and injury level was controlled by altering the velocity of this impounder tip. Each animal was administered one level of injury only (surgical sham, moderate tip velocity 4.5m/sec, or high tip velocity 6.0m/sec) and monitored for cognitive and motor deficits for up to 3 weeks following injury. Analysis of fine motor control included a beam-walking task in which the animal was required to traverse a raised, narrow wooden beam 10mm wide and 90cm long with minimal foot-faults. This was quantified by counting the number of right hindpaw faults, defined as the loss of contact of toes with the horizontal surface of the beam, over a total of 50 steps. In addition, the presence or absence of forepaw flexion and turning behavior while suspended by the tail was assessed and scored on a scale of 0-5. Learning and memory deficits were investigated using two different water maze training protocols. Deficits in reference memory, the longer-term storage of information pertinant to all trials, was determined using the standard Morris maze version in which each mouse was given 16 trials (30 min inter-trial separation) over 4 days and required to find a submerged platform whose position remained constant over this time period. An improvement in escape latency over days was taken as being indicative of intact reference memory function. In contrast, working memory was evaluated by administering 4 pairs of trials on each day, with the platform placed in any one of 4 positions for each trial pair (5 sec between each trial of the pair with a 4 min inter-trial separation). An intra-pair improvement in escape latency was taken as being indicative of intact working memory function.

Sham-operated animals performed well in all motor tasks, exhibiting no forpaw flexion/turning behavior or deficits in beam walking. However, animals which received either moderate or severe CCI were impaired in all four tasks, with the most pronounced deficits exhibited by the severely-injured group. A turning behavior coupled with a mild-to-moderate forepaw flexion was observed in moderately-injured mice for the first 3 days following cortical impact. In contrast, a pronounced forepaw flexion and turning behavior was evident over the same period in the severely-injured animals, with evidence of deficits remaining up to 7 days post-injury (Figure 1). In the beam walking task, both injury groups were severely impaired over the first 3 days, but began to show signs of recovery at seven days post-impact. However,

when the mice were retested at 2 and 3 weeks, the moderately-injured animals were shown to gradually recover to almost control levels whereas the severely-injured animals maintained a significant deficit (Figure 1).

Both moderate and severe injury levels produced a pronounced deficit in the ability of the mouse to learn a standard water maze paradigm when compared with sham-operated animals. This deficit was most evident in the severely-injured animals which invariably appeared to have great difficulty recalling information from previous experience in the maze. However, by the fourth and final training day, the performance of all animals was greatly improved, with the moderately-injured mice escaping to the platform in times comparable to those for the sham-operated controls. Nevertheless, when memory for the position of the platform was assessed with a standard probe trial 4 days following the final training session, sham animals markedly outperformed injured animals at both injury levels (Figure 2). This supports similar reference memory deficits which have been previously demonstrated for the rat. Preliminary data also suggests that working memory is impaired by CCI in the mouse. Sham-injured animals rapidly learned to locate the platform position on the second of each pair of trials whereas injured animals were impaired, with the severely-injured group once again exhibiting the most pronounced effect. Anterograde reference memory, as assessed by comparing interday differences for the first trial per pair in each group, was once again affected by CCI.

Conclusions

The results presented demonstrate a pronounced impairment in both motor and cognitive abilities in the mouse following CCI, with fine motor control deficits persisting up to 3 weeks following injury. Deficits in the two water maze tasks indicate a disorder in anterograde reference memory, in addition to impaired working memory, both of which are consistent with mnemonic impairment observed following TBI in the human. During the next grant year, we will investigate newly developed pharmacological strategies with regard to cognitive and motor improvement after trauma. Mice with genetic manipulations (transgenics, knock-outs, point mutations) will be evaluated to determine the potential roles of specific gene products in producing neuronal loss after brain trauma. Magnetic resonance imaging techniques - diffusion and perfusion imaging, high resolution imaging - will be performed in this experimental model using a 7.0T animal magnet to better define anatomic correlates of injury and plasticity.

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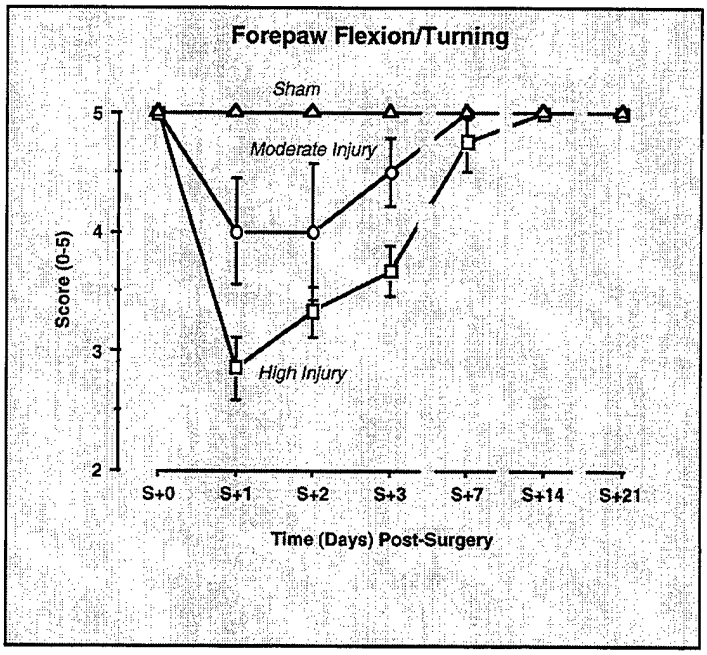

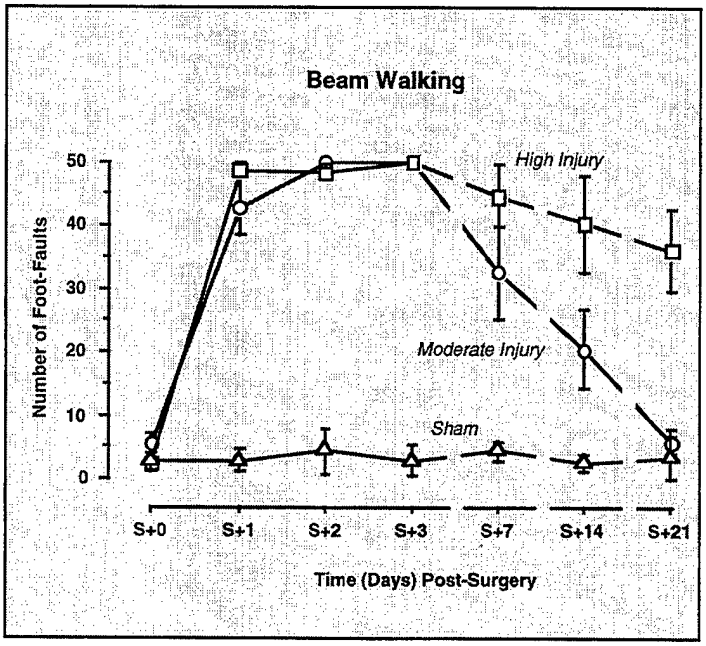

Figure 1: Effect of CCI on motor performance in the C57B6 mouse.

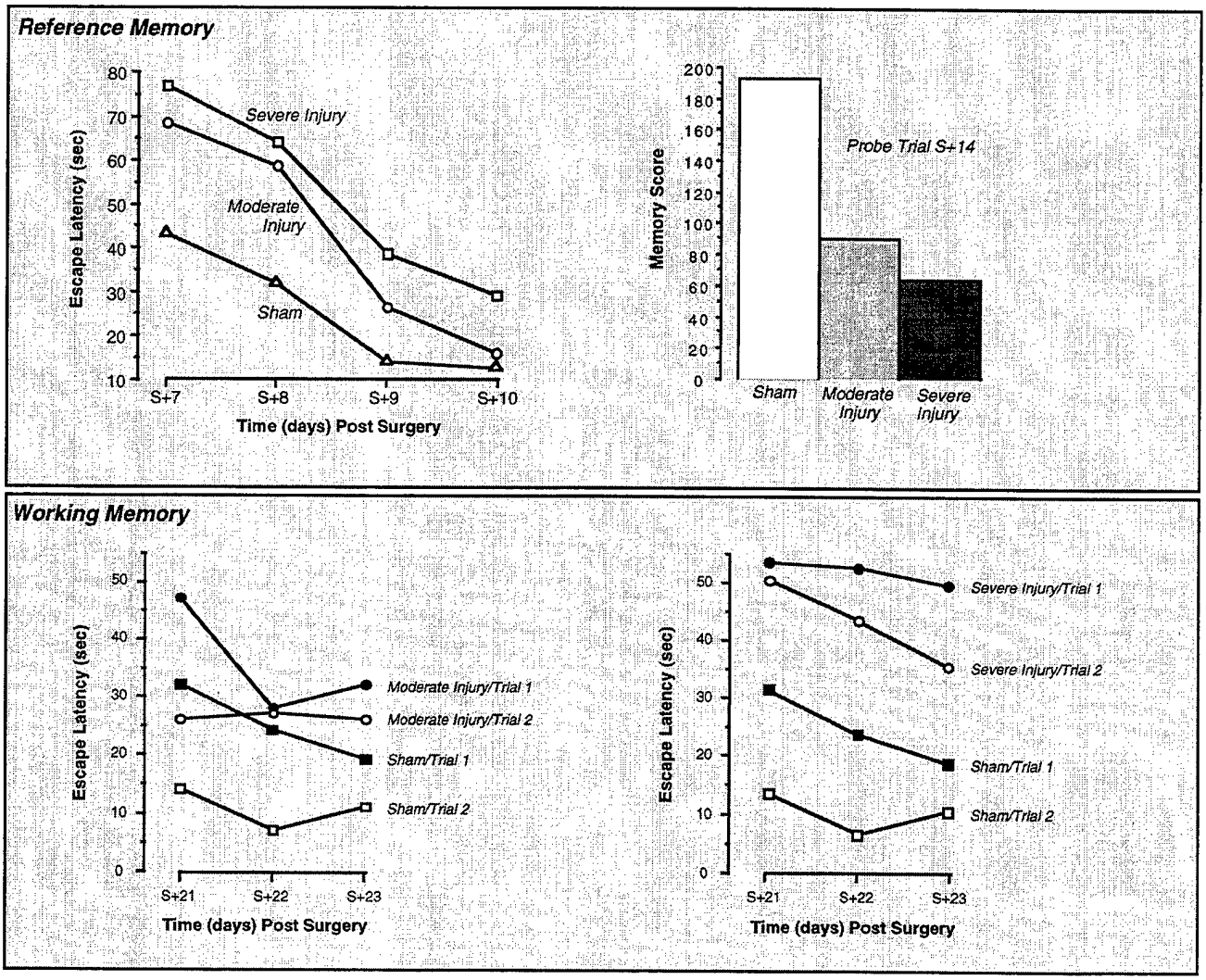

Figure 2: Effect of CCI on C57B6 mouse performance in 2 water maze learning tasks.

Project 4: Development of Novel TRH Analogs as Potential Neuroprotective Agents in Neurotrauma

Introduction

Thyrotropin-releasing hormone (TRH) improves neurological recovery after traumatic CNS injury (Faden, et al, 1981), and reverses cognitive deficits associated with CNS lesions (Ogasawara, et al, 1996) or electroconvulsive therapy (ECT) (Kahn, et al, 1994). However, therapeutic administration of the hormone is limited by its short half-life and inability to readily gain access to the CNS through the blood brain barrier; in addition, TRH treatment may be associated with undesired endocrine and autonomic effects (Faden, 1989). Because of these constraints, a variety of TRH analogs that are relatively resistant to degradation have been developed. Depending on the type of structural modification involved, these compounds are reported to have differential association with endocrine, autonomic, or centrally mediated effects, presumably through as yet unidentified homologous receptor subtypes (Feuerstein, et al, 1984).

Structure-activity studies of the various analogs in CNS injury suggest that an intact C-terminus is necessary for neuroprotection (Faden, et al, 1988), and that modification of the imidazole ring can reduce endocrine or autonomic actions without reducing neuroprotective effects (Faden, et al, 1993). Recent studies in animal models of learning and memory (Drago, et al, 1991 and Stwertka, et al, 1992), and in individuals with Alzheimer's disease (Parnetti, et al, 1995) also support cognitive enhancing properties for TRH analogs.

In collaboration with Dr. Alan Kozikowski, we have recently developed a series of novel TRH analogs with modifications intended to limit endocrine and autonomic effects, extend half-life, increase CNS permeability, and retain neuroprotective properties. These compounds are being screened for their potential neuroprotective activity when administered acutely in a clinically relevant trauma model. In addition, potential cognitive enhancing effects of the analogs will be assessed independently in naive animals and in those with learning and memory deficits caused by traumatic brain injury.

Methods and Results

The following analogs have been synthesized: 1-ARA-97a, 1-ARA-98a, 2-ARA-66b, 3-ARA-57a, 2-ARA-29a, 2-ARA-05a, 2-ARA-17b, 2-ARA-60a, 1-ARA-96a, 3-ARA-53b, 3-ARA-53a, 2-ARA-35b. Preliminary binding studies, performed by Dr. Marvin Gershengorn (Cornell Medical Center), show that these compounds have a range of affinities at TRH receptors which bind 3MeHisTRH with high affinity. These data, along with mechanistic potential, have been considered together with information obtained from preliminary work on solubility, tolerance and mortality to select a limited group of analogs that will be more extensively examined. These include: 2-ARA-29a, 3-ARA-57a, 2-ARA-35b and 3-ARA-53a.

All trauma studies utilize a fluid-percussion injury model that has been extensively characterized with regard to biochemical, behavioral and histological changes, as well as pharmacological manipulation (Faden, et al., 1989 and Faden, et al., 1993). Acute pathophysiology studies will use the following outcome measures: 1) MRS/MRI assessments of alterations in cerebral metabolism, blood flow, pH and edema; 2) behavioral recovery, including mortality, graded performance on motor tests (forelimb flexion, lateral contrapulsion, angle board) as well as learning and memory (Morris water maze) tasks; 3) delineation of the extent of the lesion and neuronal survival using high resolution MRI in conjunction with histological methods. The acute studies are in progress and compare the effect of the new compounds to negative (vehicle) and positive controls.

Studies examining the restoration of trauma-induced cognitive dysfunction are to begin in early 1997. These experiments will utilize a series of learning and memory tests (Morris water maze, passive avoidance, T-maze) to assess nootropic effects of the analogs. Drugs that show neuroprotective and/or cognitive enhancing effects will be fully characterized with respect to CNS permeability, half-life and endocrine or autonomic activity.

Conclusion/Significance

Completion of the described studies will permit comprehensive evaluation of potentially novel therapeutic agents that may prevent or limit the acute tissue damage that follows CNS trauma. In addition, the potential cognitive enhancing/restorative effects of these compounds may have substantial potential for treatment of cognitive dysfunction following acute CNS injury or neurodegenerative disorders.

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SHERIDAN L. SWOPE, PH.D.

Introduction

Neurotransmitter receptors are of primary importance in synaptic transmission and are potential targets for regulation. Modulation of the function, expression, or density of receptors could have a profound effect on synaptic efficacy and thus mediate plasticity. Accumulating evidence supports a role for phosphorylation in the regulation of synaptic transmission (Swope et al., 1992). Protein tyrosine kinases are a unique class of kinases initially found to mediate cell transformation and proliferation. However, protein tyrosine kinases are also involved in differentiated cell function. In fact, protein tyrosine kinases are highly expressed in the brain, being present both pre- and postsynaptically, suggesting their importance in the regulation of synaptic activity. Our long-term goal is to study the role of protein tyrosine kinases in synapse formation and function.

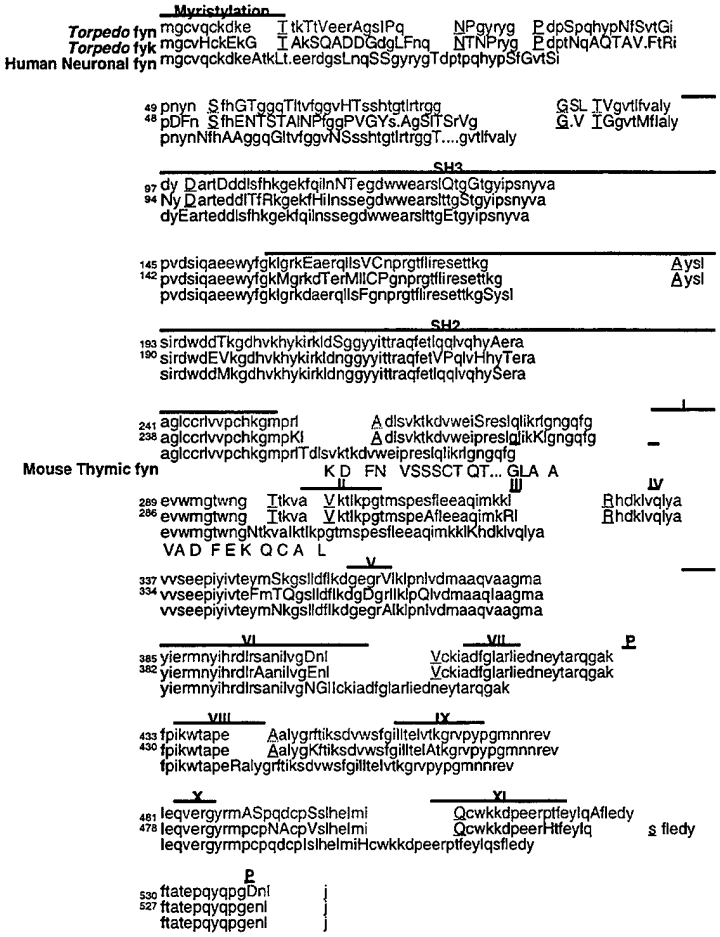

Fig 1. **Complete amino acid sequences of Fyn and Fyk**. The deduced amino acid sequences of Fyn and Fyk are indicated. For each clone, amino acid identities with human neuronal Fyn are indicated by shading. Amino acids of nonidentity with neuronal Fyn but identity between Fyn and Fyk are indicated by underlining. The divergence between the neuronal and the alternatively spliced thymic form of Fyn is shown. Conserved protein tyrosine kinase subdomains are indicated by horizontal bars: SH3 and SH2, src homology domains 3 and 2; P, putative phosphorylation sites; I-XI, conserved subdomains. The amino acid postions in the sequence of each clone are indicated on the left.

Much of the current understanding of synapses originates from studies of the neuromuscular junction (NMJ). The nicotinic acetylcholine receptor (AChR) is the ligand gated ion channel that mediates rapid postsynaptic depolarization at the NMJ. Because of its abundance in the electric organs of *Torpedo californica*, the AChR is the best characterized neurotransmitter receptor and has served as a model to elucidate the structure, function, and modulation of neurotransmitter receptors and ion channels. The AChR is phosphorylated on tyrosine residues both *in vitro* and *in vivo,* and this tyrosine phosphorylation is correlated with a modulation of the rate of receptor desensitization. In addition, tyrosine phosphorylation of the AChR and/or other postsynaptic components is involved in the nerve induced clustering of the AChR during synaptogenesis at the NMJ. Furthermore, protein tyrosine phosphorylation mediates the effect of acetylcholine receptor inducing activity (ARIA) to increase AChR transcription by nuclei underlying the synapse. Our interest has been to identify protein tyrosine kinases that are expressed postsynaptically at the NMJ which function to regulate the AChR.

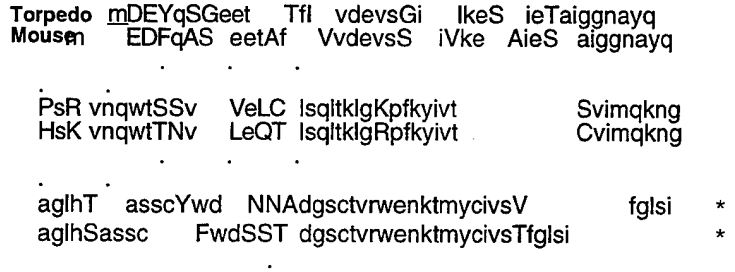

Fig 2. **Complete amino acid sequences and homology between Torpedo and Mouse Tctex.** Using the yeast-two hybrid system with the unique domain to Fyn as a probe, a positive clone was identified and dideoxysequenced. The deduced translation product of the Torpedo clone and its homology to mouse Tctex is shown. Amino acid identities are indicated by shading.

We have identified two Src like kinases, Fyn and Fyk, that together comprise the predominant protein tyrosine kinase activity in the AChR enriched postsynaptic membrane of *Torpedo* electric organ (Fig. 1; and Swope and Huganir, 1993). Fyn and Fyk are also present in skeletal muscle and brain of *Torpedo* and mammals. In skeletal muscle, Fyn and Fyk are regulated by both differentiation and denervation. These two kinases associate with the AChR via a binding of their src homology 2 (SH2) domains to the tyrosine phosphorylated δ subunit of the receptor (Swope and Huganir, 1994). In addition, Fyn and Fyk phosphorylate the receptor *in vitro* (Swope and Huganir, 1993). These initial studies suggest that Fyn and Fyk are important regulators of synaptic function at the NMJ and in the central nervous system.

Laboratory Development

Over the last year, we have established a wet laboratory to perform biochemical, molecular biological, and cell biological experimentation. In addition, appropriate personnel have been recruited. Presently, our laboratory is comprised of the following personnel: Dr. Ali Mohamed, a postdoctoral fellow with biochemical and molecular biological experience in the area of protein phosphorylation; Mr. Tao Mou, MS, a research assistant with a master's degree in Biochemistry from Georgetown University Medical Center and extensive molecular biological experience; Ms. Mavis Wu, MS, a summer student with a masters degree in Physiology from Georgetown University Medical Center. The efforts of these researchers will enable our laboratory to attain its research goals. We have also been successful at obtaining extramural funding from the National Institutes of Health, National Institute of Neurological Disorders and Stroke in the form of an R29 or First Award.

As a new laboratory at the Georgetown Institute of Cognitive and Computation Sciences, the aim of our current research is to continue to test the hypothesis that Fyn and Fyk are involved in synapse formation and function at the NMJ by regulating the AChR. Furthermore, the molecular mechanisms by which these two kinases act to regulate synaptic transmission are being investigated.

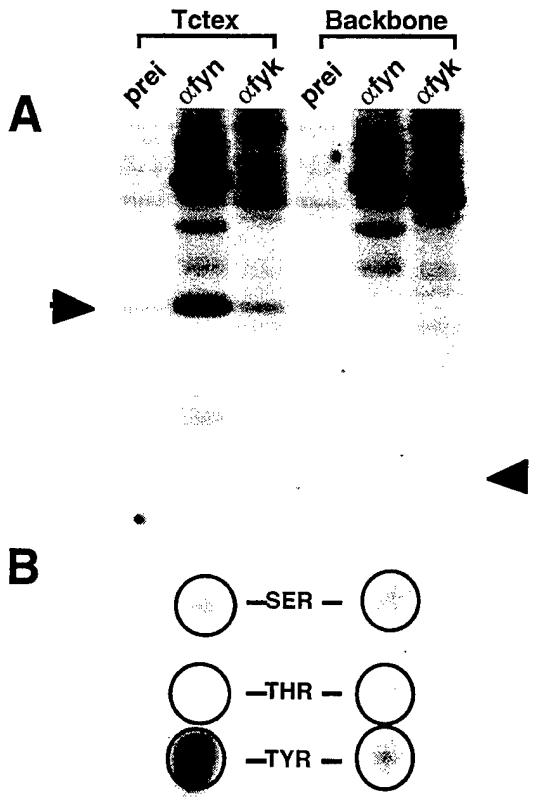

Fig 3. **Phosphorylation of Tctex Fusion Protein by Fyn and Fyk. A**, Torpedo electric organ postsynaptic membranes were solublilized, and immunoprecipitated with the anti-Fyn (αFyn), anti-Fyk (αFyk), or preimmune (prei) serum as described (Swope and Huganir, 1993). The immunoprecipitates were incubated with 1μg Tctex or backbone fusion protein and [γ-^{32}P]ATP under phosphorylating conditions at 30°C for 30min as described (Swope and Huganir, 1993). Phosphorylation was analyzed by SDS-PAGE and autoradiography. Molecular weight markers are as indicated. **B**, Phosphorylated Tctex fusion protein that had been incubated with Fyn or Fyk, as in A, was subjected to phosphoamino acid analysis as described (Swope and Huganir, 1993). The postions of phosphotyrosine (TYR), phosphothreonine (THR), and phosphoserine (SER) are as indicated.

Identification of Primary Extracellular Activators of Fyn and Fyk

As a first step to attain our research goal, we plan to identify primary extracellular factors that regulate Fyn and Fyk activity. The ability of a series of known candidate factors including agrin, ARIA, and bFGF to stimulate Fyn and Fyk activity will be examined in muscle cells in culture and by using molecular biological and biochemical approaches. Presently, we have demonstrated that an antiserum generated to the C-terminus of the putative agrin receptor, MuSK does recognize MuSK in postsynaptic membranes of *Torpedo* (unpublished results). We are using this antiserum to test for an association of MuSK with Fyn and Fyk using coimmunoprecipitation and affinity chromatography experiments

Clarification of the Signal Transduction Cascade Involving Fyn and Fyk

We are also in the process of characterizing the signal transduction pathway(s) by which Fyn and Fyk act to regulate synapse formation and function by asking: what are the identities of cellular components that interact directly with Fyn and Fyk? These on-going studies will be performed using coimmunoprecipitation techniques, fusion protein affinity chromatography, and the yeast two-hybrid system. The yeast two-hybrid method is excellent for discovering functional interactions between two proteins. The yeast GAL4 protein is a transcriptional activator consisting of a DNA binding domain and a transcriptional activation domain. This system uses two different vectors each containing one of the GAL4 domains. Neither domain alone has the ability to activate transcription. However, if the two domains are brought together, for example via protein-protein interaction, transcriptional activation is restored. By fusing a known protein such as a fragment of Fyn and Fyk to the binding domain, proteins that interact with that region of the kinase can be identified from a cDNA library fused to the activating domain. This method has become generally applicable for the cloning of target proteins based on protein-protein interaction including protein kinase substrates.

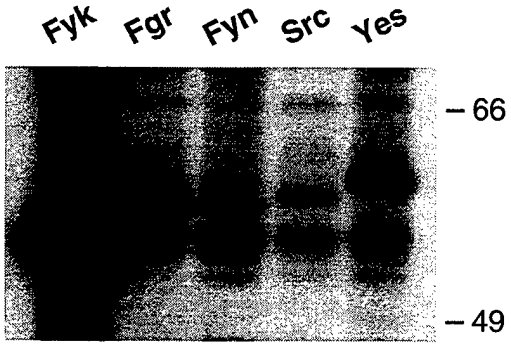

Fig 4. **Expression of Fyk in Rat Brain.** Membrane proteins (50ug) prepared from rat brain were solubilized at 0.125mg/ml, immunoprecipitated with anti-Fyk, anti-Fgr, anti-Fyn, anti-Src, or anti-Yes serum as indicated and incubated under phosphorylating conditions at 30°C for 30min in the presence of [γ-32P]ATP followed by analysis by SDS-PAGE and autoradiography for 1.5h.

Using the yeast two-hybrid system, we have we successfully identified a molecular component of *Torpedo* electric organ that interacts with the unique domain of Fyn. This 13kDa component was homologous to a previously identified mouse sterility factor, Tctex-1 (Fig 2). *Torpedo* Tctex-1 (Tctex) was detected in the cytosolic fraction of electric organ using antiserum generated to a fusion protein derived from the *Torpedo* clone (unpublished results). In addition to the association in yeast, Tctex bound a protein tyrosine kinase activity which subsequent immunoprecipitation demonstrated to contain Fyn (unpublished results). The sequence of *Torpedo* Tctex indicated a putative tyrosine phosphorylation site at Tyr4. In fact, we found that Tctex fusion protein could be phosphorylated *in vitro* by immunoprecipitated Fyn (Fig 3A). Phosphoamino acid analysis demonstrated that the phosphorylation was on tyrosine residue(s) (Fig 3B). These results suggest that Tctex may be a substrate for Fyn at the NMJ. Other workers have demonstrated that mouse Tctex-1 is highly expressed in sperm tails and oocytes where it has been suggested to be associated with cytoskeletal elements. Thus, Tctex may be involved in the proposed anchoring of postsynaptic components at the NMJ by cytoskeletal elements, a process that involves tyrosine phosphorylation of postsynaptic components. Determining the function of Tctex at the NMJ will be a focus of ongoing experiments in our laboratory.

Determination of the Physiological Roles of Fyn and Fyk

A final goal of the laboratory is to determine the physiological role of Fyn and Fyk in synapse formation and function. To do so, a short term aim is to obtain evidence as to the importance of Fyk, a novel protein tyrosine kinase, in mammalian brain. Recently we have demonstrated that Fyk, in rat brain, represented a protein tyrosine kinase activity of molecular weight 53kDa which was distinct from other Src-like kinases including Fgr (59kDa), Fyn (59kDa), Src (60kDa), and Yes (62kDa) (Fig 4). In addition, Fyk was found to be the most abundant Src like kinase activity in rat brain (Fig 4). Our present aim is to molecularly clone the mammalian homologue of Fyk. Using the catalytic domain of *Torpedo* Fyk, we have identified a series of clones from mouse brain and spleen phage libraries. These clones are presently being sequenced at the DNA level and identity with *Torpedo* Fyk determined. The mammalian Fyk and Fyn clones will be used in heterologous expression systems to investigate the physiological roles of these two protein tyrosine kinases in the regulation of AChR phosphorylation, clustering, expression, and channel kinetics.

Clarification of these basic molecular processes are relevant to an understanding of synaptic transmission in healthy people as well as those afflicted with neurological and neuromuscular diseases.

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OTHER GICCS SPONSORED RESEARCH

ROBERT S. LEDLEY, D.D.S.

Progress Report I
"Texture Analysis for Scene Segmentation"
Phase II
PI: Dr. Robert S. Ledley

I. Background

Texture analysis is an area which is blooming in the image processing arena. This is due to the fact that scientists are discovering that the spatial-structure of intensity variation is important for characterizing image regions. In the neurophysiological arena investigators have been examining specific cells in the cortex which appear to be sensitive to various textures. The idea that specific basic texture elements are used to determine the structure of visual textures was suggested by Julez and Bergen (1983). These basic texture elements, termed Textons, were investigated in the past but have been challenged (Voorhees and Poggio, 1988).

There is evidence that the mammalian visual system relies more on the orientation characteristics of texture components than the space-frequency characteristics when discriminating between textural components within classical Receptive Field (RF) structure (Geri, Lyon and Zeevi, 1995). Integration fields, which have much larger sized input areas, however, are less sensitive to stimulus orientation and more sensitive to spatial frequency (Li and Li, 1994). Complex dot patterns perceptually "popped-out" of an image and complex cells have been found to respond to such patterns (DeWeerd, Sprague, Vandenbussche and Orban, 1994).

The mammalian visual system also appears to concentrate on macrotextures rather than microtextures when both exist in a pattern. Macrotextures are larger scale subtextures with lower spatial-frequencies that comprise a large part of the texture, whereas microtextures are smaller scale subtextures that are higher in frequency or are localized to only one portion of the texture. In scale-space terms the macrotextures do not lose much of their structure with smoothing whereas the microtextures do. It has been shown that when subjects are asked to discriminate patterns which contain both macro- and micro- textures, the visual system attends to the macro-structure of the pattern and ignores the micro-structure of the pattern. This suggests that the visual system might first determine the regions of interest size and adjusts the resolution of feature detectors to best analyze the textured pattern (Dehaan et al., 1994).

Although Hofmann and Hallett (1993) showed that simulated neural network models of the visual system using symmetric (even) Gabor functions fit psychophysiological data better than models using antisymmetric (odd) Gabor functions, the authors determined that the network required antisymmetric (odd) Gabor functions to discriminate between mirror-image pairs of textures.

A comparison of three statistical-type and one space-frequency method of texture analysis showed that cooccurrence and fractal texture descriptors out performed Markov Random Field and Gabor function based measurements (Ohanian and Dubes, 1992). However, a recent study suggests that a spatial-frequency texture descriptor based on the frequency of local extremas perform slightly better than cooccurrence and Gabor function derived measurements (Strand and Taxt, 1994). The literature is filled with new texture descriptors but only a few studies have made the comparisons between methodologies. The existing comparison studies are limited by the type of textures evaluated, with the natural Brodatz textures being the most common type.

The current trend is to implement an approximation of the Gabor function using wavelets. However, studies using Gabor-type functions for texture identification in image analysis have not demonstrated impressive results. This many be due to the fact that only symmetric Gabor symmetric functions are used for texture analysis.

II. Classes of Texture Analysis

Our texture analysis model of the visual cortex is an extension of both image processing methods and neurophysiological models. This model has applications as a model of the visual cortex and as a tool for texture recognition in image processing, and it overlaps several categories of traditional texture analysis

methodology. For example, one class of texture analysis methods use spatial-frequency characteristics derived from intensity information of an image region to define texture characteristics. An archaic implementation of this approach would be calculating the magnitude of the Fourier power spectrum, where as a more modern example of this approach would be to derive texture characteristics through wavelet decomposition. Since Gaussian Derivatives (GDs) are wavelets, they are definitely related to this class of texture measurement methods, however, GDs also are related to other classes of texture measurements.

Another class of texture analysis methods extract statistical texture properties using theoretically defined mathematical procedures. These methods measure common characteristics of a distribution, and are usually applied to histograms of intensity values over an image region. As mentioned earlier, the first four GDs represent the first four moments of the Gaussian distribution: mean, variance, skewness and kurtosis. Thus, applying these spatial-frequency filters to a gray-level intensity image region provides statistically meaningful information concerning that region, assuming the image signal is distributed in a Gaussian manner.

Finally, a third class of texture measurement methods extract structural information from the an image region. This class of texture measure is probably the most commonly used. An examples of this method might be the application of a convolution mask to the entire image. Such a mask measures the commonality between a predefined pattern and image intensity patterns, and is rather popular in radiological analysis of chest and breast images when combined with a neural network. Other examples of texture measurement methods in this class include those which use a cooccurance matrix, Markov and autoregressive models, and the texture spectrum. These latter models all provide a likelihood of the spatial interrelationship between image intensities. The GD RFs extract information which can be considered line and edge information.

Simple and Complex Cells Combine to Produce Shift-Invariant Feature Detectors

As discussed above, GDs are well suited to be used as feature detectors. The first GD closely approximates the Canny edge detector, an edge detector theoretically designed to optimally locate an true edge accurately. The second GD resembles an oriented bar detector of the visual cortex. In frequency space, the first four GDs fit the spatial frequency profiles of the recorded RFs.

Frye and Ledley (in press) have pointed out that the response of a feature detector appears to be dependent on the phase of a periodic underlying pattern. Using the (Discrete Cosine Transform (DCT) coefficients as an array of feature detectors, they showed that the coefficients change periodically as a periodic stimulus is shifted along the horizontal or vertical axis. The DCT is usually applied to adjacent image blocks without regard for the structure of the underlining image intensity patterns. Application of the DCT in a block like fashion does not give any information regarding the underlying pattern phase shift relative to the DCT feature detectors. A different set of DCT coefficients will arise for each phase shift of the an underlying periodic pattern. Thus, it is difficult to define a unique set of DCT coefficients of the phase shift if the pattern is not taken into account. However, if this phase shift dependency of the pattern is eliminated, only one value of a texture measurement of a pattern will exist regardless of the pattern's phase shift.

In order to allow shift invariant texture recognition, these researchers derived DCT-based shift-insensitive texture measures. The advantage of these derived texture measurements is demonstrated by texture classification. Since the shift-insensitive texture measurement values remain the same regardless of a texture's phase shift, a unique set of values can be assigned to each texture. Each unique set of values can then be compared to an unknown sampled texture regardless of the sampling region's relative phase shift to the texture.

This shift-dependent response of feature detectors can be generalized to any spatial-frequency filter which does not provide measurement of the signal phase. For example, a maximum response would be elicited from a RF line detector if the image of a line is placed at the center of the RF. However, a near minimal response would be elicited if the line image was placed at the inhibitory zones located slightly to the left or right of the RF center. An intermediate response would be elicited if the line image was placed in between the RF center and the RF inhibitory zones or beyond the inhibitory zones. This RF response behavior creates a continuum of responses, and if the line image characteristics, such as gray level magnitude, line orientation, line length and line width are known, then the RF response could provide information concerning the distance of the line from the RF center. However, other characteristics of the line image influence the RF response.

There are several response properties of a RF line detector that are assured the center of a line is moved to every horizontal and vertical RF position. This is analogous to considering the properties of a set of RF line detector responses to all possible phase shift of a pattern. If the line image corresponds to the line detector's tuned-characteristics, when the center of the line detector and the image are aligned perfectly, the absolute maximum line detector RF response will be elicited. If the tuned-characteristics of the line detector and the line image are not the same the maximum response of the RF line detector will be elicited where they become most similar.

The complex cells of the visual cortex are known for their position invariant response to visual stimuli (Kandel, 1991). For example, the visual field over which a complex cell responds to a visual stimulus of optimal orientation and spatial frequency is much wider than the simple cell. Thus, the RF of the complex cell is much harder to define since it does not correspond to a specific optimal pattern at one portion of visual space like the simple cell. The RF of a complex cell is analogous to several identical RF feature detectors placed adjacent to one another. This response pattern is what led Hubel and Wiesel (1962) to hypothesize that the complex cell processes inputs from simple cells with identical RF tuned-characteristic placed in adjacent visual field locations.

But how does the position invariant response of the complex cell relate to the responses of the simple cells which provide its input? Each of the simple cells which provide input respond to the underlying image along a continuum which depends on the relative phase shift between the simple cell's RF and the underlying pattern. After the output of these simple cells are processed by the complex cell, the complex cell produces an output which appears as if the underlying pattern is optimally placed relative to a RF feature detector. As mentioned earlier, an underlying pattern evokes the optimal response from a feature detector when it is positioned at the RF center, and the optimal response is the maximum response that can be elicited from the feature detector. Thus, the complex cell finds the maximal response of all simple cell inputs and provides that value as its output.

The Neocognitron was developed as a model for visual pattern recognition and has been successfully applied to handwriting character recognition. Fukushima (1982) developed this network using a hierarchial architecture of layers which contained simple and complex cells. In the Neocognitron the simple cells act like feature detectors and the complex cells provided position invariance in order to reduce sensitivity to pattern deformation. The complex cells processed the simple cell input by applying a saturation function to the quantity obtained by dividing the sum of the excitatory inputs by the sum of the inhibitory inputs. Connections from complex cells of a neural network layer to simple cells of the next neural network layer were processed using a similar calculation. The major difference between simple and complex cells in the neocognitron is the modifiability of the input weights to the simple cell. This allows unsupervised learning to occur by a winner-take-all method in which the connection from the complex cell to the simple cell which is more active is reinforced.

In this study we use GD RFs as feature detectors, and non-linear functions, such as max, min, range and median, applied over a region of feature detector output. The feature detectors simulate the operation of simple cells, where as the non-linear functions simulate the operation of complex cells. This arrangement is analogous to one layer of the Neocognitron, except that it provides a much simpler approach to feature detection. The output from the non-linear functions in our model are used as input into a linear discriminant analysis function for texture classification. We show how this simple model of the visual cortex parallels the neurophysiology and can easily classify image textures.

Method

Two texture images were fabricated from the Photogear image library on CD-ROM (Image Club Graphics, Inc.) and the Brodatz (1966) natural texture album. The first texture image contained four textures and was used to determining the most appropriate number and type of GDs and non-linear functions for the visual cortical model. The second test image contained sixteen textures, and was used to determine the ability of this methodology to distinguish between multiple textures.

The test images were processed by multiple cortical arrays, with each cortical array corresponding to a two layer linear network of functions. The primary layer, also known as the simple cells layer, contains an array of GD functions with identical parameters. The secondary layer, also known as the complex cell layer, contains identical non-linear processing functions.

The architecture of a cortical array is shown in Figure 1. Each cortical array is labeled by its unique characteristics: the orientation, size and derivative order of the GD simple cells and the non-linear complex cell function.

The resulting size of a cortical array is the size of the image. Essentially this constitutes a sliding transformation since the simple cell function is slid across the image one pixel at a time and period boundary conditions are used. This process is essential for maintaining a constant resolution across all cortical areas. The size of the image region processed by the simple cell is identical to the size of the area processed by the complex cell. This is essential for the shift-invariance of the model since the texture characteristics extracted by the non-linear function from the GD function output will occur at least once within function's non-zero spatial area for a periodic stimulus.

The GD function is zero everywhere except within three spatial units of center. The function's spatial axis within three spatial units on either side of center is divided up into the number of units it is to be scaled (i.e., scale units). Each scale unit is then divided up into ten smaller units and an average over those ten smaller units is calculated. This average is used as the coefficient at that spatial location. For two dimensional RFs, each scale unit is calculated by determining its projection onto each one dimensional orthogonal function. The coefficient is derived by multiplying the values given by each one dimensional function.

The image processing operation used to apply the RF feature detectors to the image data is essentially a convolution. When applying a convolution to an image, the image and convolution mask size are typically both a power of two. In this way the convolution operator can be easily scaled and the scaling will be directly proportional to the image size. Scale-space operators are traditionally used in sizes which are powers of two. All RF in this model are square, resulting in the same vertical and horizontal sized edges.

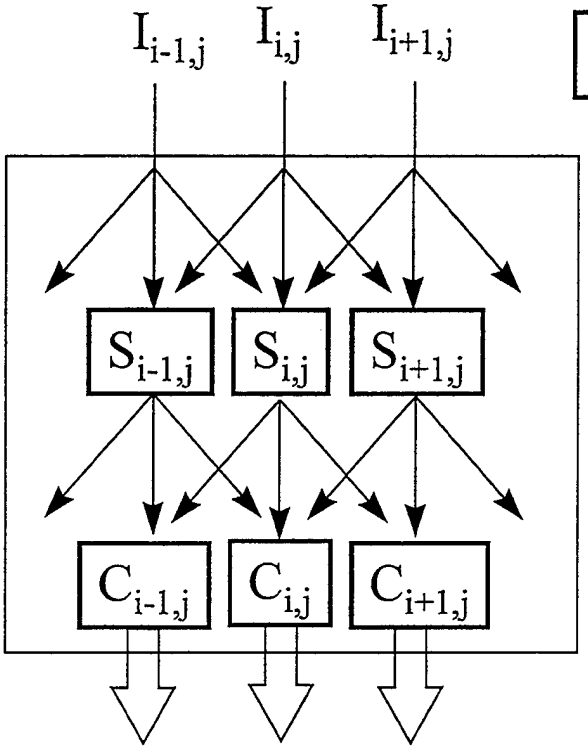
As seen in Figure 2 the cortical array with the smallest scale is associated with a simple cell whose RF size is 4 x 4 and the cortical array with the largest scale is associated with a simple cell whose RF size is 32 x 32. The fact that the horizontal and vertical size doubles for each successively larger RF is consistent with scale-space methodology. This size arrangement is also consistent with multi-resolution methodology. However, multi-resolution methodology down samples the image data with each successive application of a convolution kernel. Scale-space methodology does not change the resolution of the data, only that scale of the analysis method.

Simple cells of the cortex are known to be tuned to orientations in 30° increments. Thus, as seen in Figure 2, the two-dimensional GD is rotated to six orientations. Rotation to 180° and beyond results in even-function RFs with the identical values to RFs with 180° difference in rotation and odd-function RFs with identical negative values to RFs with 180° difference in rotation.

Several non-linear functions were used to model complex cell RFs. As mentioned earlier the maximum value that a line pattern can stimulate a feature detector will describe the correspondence between the underlying pattern and the feature represented by the feature detector. Thus, the first non-linear function used is the Maximum function.

Biologically a particular neuron cannot provide both positive and negative values to a particular output, although positive and negative values can be expressed to different outputs through excitatory and inhibitory neurotransmitters respectively. However, the mathematics of the GD functions provide them with negative values in certain portions of their RFs. These negative values could be removed by adding a small constant. However, we have taken advantage of these negative values in order to both enhance the range of the feature detectors and provide for detection of dark contrast patterns as well as the bright contrast patterns.

Figure 1: Architecture of a Cortical Area.



Cortical Array $C_{o,s,d,f}$

Derviative	d
Orientation	o
Size	s
NL Function	f

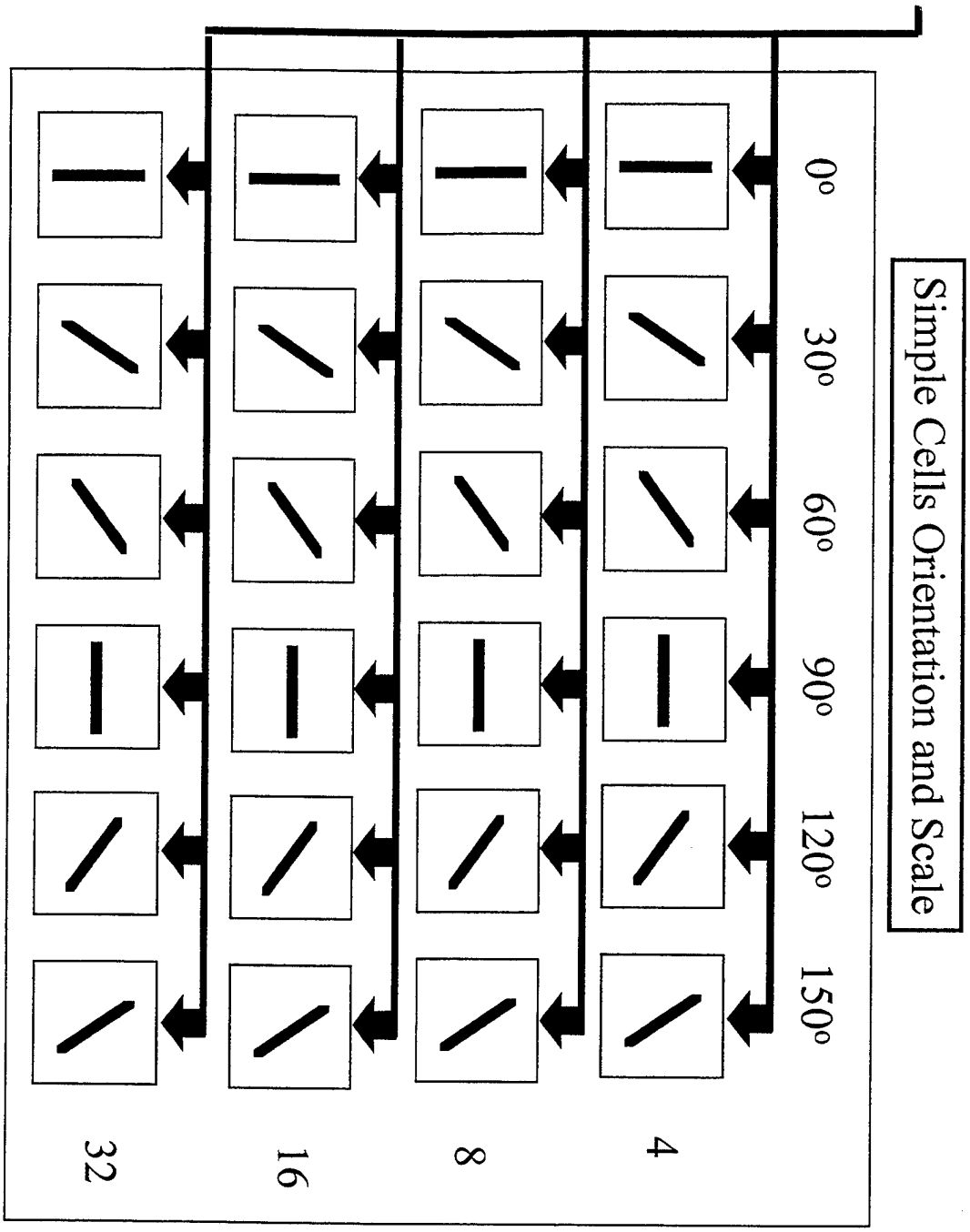
$I_{i,j}$ Intensity of Pixel at Row i and Column j

$\varepsilon(I,s,i,j)$ Creates Submatrix From Matrix I of Size s x s Centered at i,j

$$S_{i,j} = \Phi^n [\varepsilon(I,s,i,j),o,s,b]$$

$$C_{i,j} = f[\ \varepsilon(S,s,i,j)\ ]$$

Figure 2: A Set of Cortical Areas Used for Each Non-Linear Complex Cell Model



By setting the average intensity of the image to zero, all intensities larger than average will be positive and all intensities less than average will be negative. Thus instead of dark features being represented by a very small number they are represented by a large negative number. This enhances the response of the feature detectors to dark features, results in an enhanced response to bright features which have dark surrounding regions spatially matching the inhibitory zones of feature detectors, and extends the range of a feature detector's potential response. Since this scheme results in important low response values, a non-linear Minimum function was also evaluated in this study as a potential complex cell function.

Since it is expected that both the minimum and maximum values of the GD RF will be of informational significance, the range function was evaluated to determine if the magnitude of the RF response extremes could be used instead of the absolute extreme values. The range is a non-parametric statistical function which is usually used in conjunction with the median. Thus, the median was also evaluated as a non-linear complex cell function.

Gabor functions are the closest shaped basis function to GDs that have been used for texture analysis. Image processing studies either using Gabor functions as convolution kernels or the Gabor-type Wavelet analysis only use an even-type Gabor function. Hofmann and Hallett (1993) investigated the processing of visual data using Gabor function networks designed to simulate visual cortical processing. They found that using even-type Gabor functions, psychophysics data was simulated better in their model than when odd-type Gabor functions were used. Studies have only used one type of even or odd function and have not attempted to simulate RF shapes with more than two zero crossings. Initially we used only the first two order GDs so that the results are comparable to other studies. However, in order to investigate methods for improving the classification of textures for the sixteen texture image we investigated using the first six order GDs.

Traditionally a orthogonal Gaussian envelope is convoluted with the two-dimensional RF of a Gabor or GD function. This configuration best simulates the shape of the RF. In order to investigate the purpose of this Gaussian envelope we varied its size from non-existent (i.e., 0.00) to half of the width of the GD function (i.e., 4.5) in 1.5 space unit increments.

Two composite texture test images were constructed to test the applicability of our cortical model to classify textures. Before the composite images were constructed, the intensity and contrast of each texture was adjusted by setting the mean and standard deviation of the image histogram to 128 and 64 respectively. This equalized each texture with respect to its first and second statistical distribution moments. In this way, these textures cannot be distinguished based on global mean and standard deviation measures. It is unclear in many scientific articles whether such an equalization of the intensity and global contrast is performed. Failure to take this step can bias experiments, leading to non-repeatable results and invalid conclusions.

Test Image 1 (TEXT4; Photogear Square) was composed of four medium resolution (266 ppi) textures chosen from the photographic natural texture Photogear image library on CD-ROM (Image Club Graphics, Inc.). The textures consisted of one texture of crumpled paper (paper_01: Texture One), one marble texture (marbl_04: Texture Two), and two wood textures (wood_01: Texture Three; wood_02: Texture Four). Each image was converted from TIFF format to PCX format and removed of color by reducing it to grayscale. A 128 x 128 pixel portion of each resulting image was selected and saved. These four images were combined into one image such that each subimage was positioned in each of the four quadrants of the resulting square image. Contaminated TI1 images were produced by adding values sampled from either Gaussian or Uniform distributions. The distribution means were set to zero and the standard deviations were set to either 32 or 64 intensity units

Test Image 2 (TEXT16; Brodatz Textures) was composed of natural textures in the Brodatz texture album. These textures are more common to texture processing algorithms. Sixteen of the common natural textures were digitized at a resolution of 100 by 100 dpi and stored as PCX image files. Regions of size 128 by 128 were extracted from the textures of Pressed Cork (#4), Woven Aluminum Wire (#6), Long Island Beach Straw (#15), Herring Bone Weave [Large Scale] (#16), Herring Bone Weave [Small Scale] (#17), Raffia Weave (#18), Woolen Cloth (#19), French Canvas (#21), Reptile Skin (#22), Beach Sand (#28), Beach Pebbles (#54), Handwoven Oriental Rattan (#64), Wood Grain (#68), Pigskin (#92), Fur Hide of Unborn Calf (#93), and Plastic Bubbles (#112). These extracted textures were combined by arranging them in checkerboard pattern.

A multiple-group discriminant function based on the complex cell output was derived for each image. Within-group mean corrected sums-of-squares matrixes were computed from randomly selected image positions. The selection covered approximately 10% of each horizontal image line in order to ensure that larger image regions were not undersampled. A total mean corrected sums-of-squares matrix was then

computed for each image and the between-group corrected sums-of-squares matrixes were derived. The eigenvalues and eigenvectors were then computed for the $W^{-1}B$ matrix and all significant eigenvectors were retained (Dillon and Goldstein, 1984). Discriminant functions derived from these eigenvector classify each pixel by determining its closest group centroid. The classification accuracy was derived by applying this function to randomly sampled image positions. The positioned were determined as described above.

Results

In order to determine the most appropriate initial corical texture processing model, the TEXT4 image was processed using (a) complex cells with all the non-linear function mentioned above and their appropriate combinations, (b) the four different manipulations of the Gaussian Envelope, and (c) with image noise added. The output from the simple cells were also used to classify output under the conditions specified by (b) and (c). The first two GDs were used as simple cell feature detectors. Table 1 points out that discriminant functions using simple cell output (i.e., original values) classify textures near chance level. Using the minimum or maximum non-linear complex cell function output, the discriminant function appears to classify the textures well and the classification appears to be robust to image corruption from noise. Combining the output from two cortical arrays of complex cells using the Maximum and Minimum non-linear functions, improves discriminant classification and resistance to noise. The non-linear complex cells using the Range function produces output which can be used to classify textures almost as well as the combination of the Minimum and Maximum functions. However, classification using the Range function did not demonstrate the same robust behavior as the combination of the Minimum and Maximum functions. The Median functions does not provide output which can be used to classify textures, even when combined with the Range function.

In order to determine the contribution of the odd and even simple cells to the ability of the discriminant function to classify textures, separate discriminant analyses were derived from the output of cortical areas containing only even and odd simple cells. Image TEXT4 was used for this experiment. As can be seen in Table 2, classification rate is slightly higher for discriminant functions which use both odd and even complex cell output than either one alone. However, this effect is minimal and is most likely due to the model containing more variables rather than more descriptive variables. Since the discriminant function provided slightly higher classification using the even complex cell output, these RF functions were initially chosen to classify the textures in TEXT16.

Using even complex cell output to produce a discriminant function for the TEXT16 image produced a classification matrix which was disappointing (Total Correct Classification = 74%). To attempt to improve this classification odd complex cell output was also added to the discriminant function. This did not appear to help the classification markedly (Total Correct Classification = 75%). Thus, we investigated the influence of adding additional cortical areas with higher order GD simple cells.

Utilizing the output from eight cortical arrays utilizing the first four GDs and Minimum and Maximum non-linear Complex cell functions, a discriminant function was produced which could classify the TEXT16 well (82.3%). However, when odd-simple cells were eliminated the classification accuracy dropped considerably (75.4%). Using the first six GDs as simple cell functions did not improve classification (82.8%).

Table 1

	No Noise				Gaussian 32				Uniform 32			
Gaussian Smoothing	0.0	1.5	3.0	4.5	0.0	1.5	3.0	4.5	0.0	1.5	3.0	4.5
Original Values	24.68	30.13	26.51	27.76	27.98	25.26	27.11	26.63	30.20	26.11	24.13	26.07
Max	91.14	91.44	89.24	88.87	92.21	90.19	90.56	90.23	92.48	90.25	88.70	90.22
Min	91.53	93.06	91.80	91.95	93.01	92.12	90.11	89.86	92.54	91.73	93.24	89.99
Max/Min	94.88	94.09	94.04	92.74	94.77	92.70	93.32	92.33	94.65	94.35	94.25	93.40
Median	44.98	53.72	55.86	47.54	41.15	41.76	49.96	47.73	37.61	49.03	53.57	52.25
Range	95.09	91.52	91.82	91.74	94.44	92.32	91.92	87.71	92.89	90.13	91.68	87.84
Median/Range	93.11	92.56	91.71	90.89	94.06	90.55	91.04	91.51	93.90	90.89	93.41	90.37

Table 2

		No Noise				Gaussian 32				Uniform 32			
Gaussian Smoothing		0.0	1.5	3.0	4.5	0.0	1.5	3.0	4.5	0.0	1.5	3.0	4.5
Even	Max	88.00	78.59	89.20	89.71	79.91	88.63	80.20	87.83	89.03	83.36	88.61	90.11
	Min	91.86	92.25	91.35	89.38	90.34	89.03	89.02	90.22	87.00	90.17	91.44	90.47
	Max/Min	94.33	92.59	93.26	92.26	93.33	93.19	92.48	91.96	92.98	90.98	93.31	91.73
Odd	Max	85.96	81.16	83.60	79.94	84.93	81.70	81.56	78.39	84.93	82.40	77.42	77.55
	Min	88.06	84.46	84.67	79.78	80.70	82.20	74.27	81.75	86.68	78.65	81.50	83.03
	Max/Min	94.06	87.35	89.31	90.29	92.52	90.09	89.18	87.70	92.38	87.84	87.93	91.20

Conclusions

This study has shown that GDs can be used to classify texture when used in a model of the visual cortex. This study suggests that Minimum and Maximum non-linear functions are suitable for modeling the behavior of visual cortical complex cells. The non-linear Range function may also suitable as such a model, however, our initial studies suggested that its performance was slightly below that of using both the Maximum and Minimum non-linear functions.

The cortical model described above with the first two GD functions and Minimum and Maximum non-linear complex cells can provide output which is extremely useful for classifying textures in simple texture images and is robust to image corruption by Gaussian and Uniform noise. It appears that simple texture images can be classified well with only even-GDs, but that more complex textured images require both even and odd GDs and possibly higher order GDs. Although only GDs up to order four were useful in this study, it is conceivable that more complex textured images may require higher order GDs.

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CRAIG PLATENBERG, M.D.

Introduction

Functional MRI (fMRI) using the BOLD (Blood Oxygen Level Dependent) technique has demonstrated localized brain activation associated with regional changes in blood flow that imply neuronal activation (1,2). We performed fMRI to evaluate activation in the cerebral hemispheres in volunteers during the performance of a modified version of the Paced Auditory Serial Addition Test (PASAT) (3) as well as a modified PASAT test using visual stimuli. It was hypothesized that these tests would activate regions responsible for attention and working memory.

Methods

Seven normal right handed volunteers were studied on a 1.5 T actively shielded whole body imaging system (Vision, Siemens, Erlangen Germany) with a quadrature circularly polarized head coil. Single-shot gradient-echo EPI pulse sequences were used to acquire images. Sequence parameters were: TE = 50 msec, TR = 3 to 4 sec, flip angle = 90, FOV = 230x230 mm2, slice thickness = 4 mm, matrix size = 64x64, acquisition time per slice= 100msec. The slice positions were angled 20 (Transverse/Coronal) which allowed for coverage of the frontal, temporal, parietal and occipital regions of the brain.

The functional stimulation pattern involved a 2 minute scan of 28 images at each slice position with 7 off, 5 on, 5 off, 5 on and 6 off. The pre and post stimulation images were analyzed using a trapezoid wave function. The functional maps were obtained using a coefficient threshold of 0.65 which corresponds to a Z- score of 2 and were then overlaid on an anatomical image.

Volunteers were first presented with the auditory PASAT test, a measure of mental arithmetic. This test requires subjects to perform mental calculation (addition) on a continuous string of numbers. The subject adds the most recently presented number to one item back. The task was repeated using visual cue cards (Modified PASAT test).

Results

During the verbal PASAT test, activation is present in all subjects in the primary auditory cortex in the temporal regions. Bilateral activation is identified however there is an increased number of activated pixels in the right temporal area. During the visual PASAT test, activation is present in all subjects in the primary visual cortex in the occipital lobes bilaterally. Bilateral frontal activity in either the medial frontal or dorsal lateral prefrontal cortex is present in 4 of 7 individuals during the auditory PASAT. Lateralized left frontal activity is seen in only one subject and no one demonstrated specific isolated right frontal lateralization. During the visual PASAT test, only one of 7 subjects had bifrontal activation. Isolated lateral activity is seen in two subjects in the right frontal area and two subjects in the left frontal area. Repeat testing over a two month interval in two volunteers demonstrated consistent, reproducible activation in the same frontal areas.

Conclusions

The primary auditory and visual cortices are activated with the auditory and visual PASAT test respectively and serve as a control for our study. Activation in the frontal areas is seen in volunteers performing serial addition tasks. This activation is located in the medial frontal lobe near the frontal horn of the lateral ventricle and/or in the dorsal lateral pre-frontal cortex. No significant lateralization is observed with the auditory PASAT. Variable frontal activity is seen in some individuals during the visual PASAT. Consistent activation is noted in identical ateas when retesting a limited number of our volunteers. More robust activation is seen with verbal stimuli in our subjects than with visual stimuli.

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SARAH SPIEGEL, PH.D.

Sphingolipid metabolites, a novel class of lipid second messengers, in neurotrophin signaling

Introduction

Sphingolipid metabolites such as sphingosine, sphingosine 1-phosphate and ceramide elicit a diverse array of biological responses in variety of cell types. However, their importance in growth, proliferation and differentiation in neuronal cells has received little attention. Most of the effects of nerve growth factor (NGF), a trophic factor known to be important for the survival and development of neurons in the central and peripheral nervous systems, are mediated by p140trk, the high-affinity NGF receptor. However, more recently it has been suggested that some of its effects may involve the low affinity NGF receptor p75NTR, which has structural homology with the TNF-a receptor and activates the sphingomyelin cycle resulting in the formation of ceramide (Dobrowsky et al., Science, 265:1596, 1994).

Methods and Results

In rat pheochromocytoma PC12 cells which express both types of NGF receptors, we have shown that NGF also activates cytosolic sphingosine kinase, which catalyzes the phosphorylation of sphingosine to produce sphingosine 1-phosphate. Further studies also indicated that the activation of p140trk kinase is required for NGF-induced activation of sphingosine kinase. To further substantiate a potential role of sphingosine 1-phosphate in the pleiotropic responses induced by NGF, we examined its effects on the survival, growth and proliferation of two neuronal cell lines, PC-12 and neuroblastoma Neuro2a cells. Sphingosine 1-phosphate enhanced cellular proliferation and prevented programmed cell death of these cells induced by serum deprivation or NGF-withdrawal, as determined by genomic fragmentation assays. In contrast, ceramide, which has previously been reported to act as a differentiating agent in neuronal cells, induced apoptosis. Thus, the balance between endogenous levels of sphingosine 1-phosphate and ceramide may determine whether neurons undergo proliferation, differentiation of programmed cell death. Further elucidation of the roles of these signaling molecules may be important for understanding the biochemical mechanisms underlying neurodegenerative disorders such as Alzheimer's and Parkinson's disease, and ischemic stroke. In collaboration with Dr. Kozikowski, we have recently described the synthesis of several analogues of sphingosine 1-phosphate in which the phosphate ester function is replaced by a bioisosteric group, i. e., methylenephosphonate, difluoromethylenephosphonate, oxymalonate, or phosphorothioate (1). Moreover, we also synthesized the 1-deoxy-1-fluoro analog, which may be expected to act as a kinase inhibitor since it is bioisosteric with the natural substrate of the enzymatic reaction, but cannot be a substrate for the enzyme. Because these isosteric manipulations involve minimal changes to the structure of naturally occurring sphingosine, we anticipate that these analogs will exhibit either agonist or antagonist biological properties. We are now evaluating these synthetic compound as mimetics of sphingosine 1-phosphate in cell growth and apoptosis.

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Dr. Bavelier

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