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SUMMARY STATEMENT
(Privileged Communication)

Release Date: 11/04/2005

Application Number: 1 R21 NS055087-01

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Review Group: ZRG1 BDCN-L (90)
Center for Scientific Review Special Emphasis Panel

Meeting Date: 10/06/2005
Council: JAN 2006
Requested Start: 04/01/2006

RFA/PA: PA03-107
PCC: JACOBTNE

Project Title: Pathological synaptic transmission in ischemic hippocampus

SRG Action: **

Human Subjects: 10-No human subjects involved

Animal Subjects: 30-Vertebrate animals involved - no SRG concerns noted

Project Year	Direct Costs Requested
1	125,000
2	125,000
TOTAL	250,000

****NOTE TO APPLICANT:** As part of the initial scientific merit review process, reviewers were asked to identify those applications with the highest scientific merit, generally the top half of applications that they customarily review. At the study section meeting, those applications were discussed and assigned a priority score. All other applications, including this application, did not receive a score. Provided is a compilation of reviewers' comments prepared prior to the meeting, without significant modification or editing by NIH staff.

1R21NS055087-01 FOWLER, JOHN**CRITIQUE 1:**

Significance: The PI is proposing that there could be an overlooked, different phenomenon (ischemic return) which could be responsible for the failure of previous approaches to treating ischemia. However, how it might be responsible, is not dealt with in this application, and data indicating that it is an entity separate from the mechanisms of anoxic depolarization are not provided. Consequently, significance is difficult to determine.

Approach: This is an application aimed at investigating an ischemic phenomenon that precedes the anoxic depolarization (AD) which is widely held to herald ischemic damage. The PI defines this as “a paradoxical, transient return of evoked synaptic transmission”. This phenomenon is seen in the hippocampal slice model upon OGD. He defines the *ischemic return of evoked synaptic transmission* as a transient return of evoked synaptic potentials at the Schaffer collateral-CA 1 projection that interrupts the early suppression of synaptic transmission.

The presence of the ischemic return phenomenon in the *absence* of an AD can still be associated with ischemic injury in the hippocampal slice. The ischemic return appears to arise from an altered field excitatory postsynaptic potential (fEPSP)-to-action potential coupling. The PI hypothesizes that the ischemic return of evoked synaptic transmission results from a pathological increase in membrane and synaptic excitability that overwhelms neuronal defense mechanisms.

The PI will use electrophysiological techniques to characterize intrinsic membrane currents and synaptic properties underlying the ischemic return of evoked synaptic transmission. He will also use functional assays and histochemical techniques to characterize the ischemic return's contribution to acute ischemic injury in hippocampal CA1 subregion.

The PI does not make a compelling case that there is a causal relationship between ischemic return and ischemic damage, nor that it is relevant in the *in-vivo* situation. Assuming that there is a causal relationship, then the relevance of this to current philosophies of stroke treatment is unclear. The investigator has already stated that all clinical neuroprotection trials have failed. These trials have used antagonists of the glutamate receptors and ion channels that would also underlie the current phenomenon. Practical considerations preclude using these antagonists in a way that would selectively address the ischemic return (patients get them when they have clinical signs of stroke, whether or not they are already in the AD phase, or not). Thus, the investigator should clarify how studying the ischemic return phenomenon might translate into better approaches for future stroke treatment. The preliminary data section needs to be better organized.

In AIM1, though the PI proposes an extensive characterization of the currents, there is no attempt to determine whether the currents are carried by the same synaptic mechanisms which mediate excitotoxicity, or the same membrane currents which are responsible for AD. If so, then one could not realistically claim that the ischemic return phenomenon is distinct from the AD process. This is critical, given that if they are just 2 manifestations of the same mechanisms, then the novelty of this ischemic return phenomenon is considerably less.

The choice of the currents the PI proposes to study in sub-aim 1.2 is not justified in the context of ischemic mechanisms. However, it appears that his expectation is that these currents are not unique to the ischemic return phenomenon.

AIM2 is directed at determining whether the ischemic return phenomenon is related to ischemic injury. Presumably, if it is not, then AIM1 is debatable. Thus, AIM2 should be conducted before AIM1.

In Aim 2, the PI hypothesizes that “suppression of the ischemic return employing strategies derived from the experiments in Aim 1 will improve recovery”. However, there is no guarantee that the pharmacological approaches in Aim1 only affect the currents of the ischemic return, and not those responsible for AD. Therefore, it will be difficult to tell whether any neuroprotection seen is the result of suppression of ischemic return, or the underlying processes that eventually lead to AD.

The application is to study slices exposed to conditions that cause ischemic return with or without AD using a live-dead assay at 30,60, and 180 minutes. Generally, ischemic neurons may take longer than that to die, and this approach may under-estimate the toxicity of the insult, or the neuroprotective effect of an intervention.

Innovation: The innovation here is that the PI has detected a phenomenon in the hippocampal slice which he proposes may have been previously ignored, and which may contribute to the mechanisms of ischemic brain damage. If this is the case, then this is indeed a novel finding.

Investigators: The investigator is an Associate Professor at the Texas Tech University Health Sciences Center, Lubbock, Texas. He has a modest publication record, with 5 papers in the last 5 years. He has the equipment and resources to carry out the studies, and the expertise required.

Environment: No concerns.

Overall Evaluation: Overall, this is an application to characterize a current observed in hippocampal slices under certain conditions (OGD with 2% glucose) which may, or may not be a phenomenon distinct from the causes of anoxic depolarization. The application lacks the preliminary data to indicate the importance of the phenomenon, its mechanisms, or its relevance to ischemic damage. There is concern that strategies necessary to deal with the ischemic return phenomenon, if it contributes to damage, would be no different than those previously used to deal with AD.

CRITIQUE 2:

Significance: The core hypothesis of this application is that ischemic neuronal injury may be a two step event. The second of these, the profound depolarization linked to excitotoxic neuronal injury has been well studied. However, the PI argues that a transient ischemia-induced return of evoked synaptic transmission which precedes irreversible depolarization may also contribute to the extent of neuronal injury, and that anoxic depolarization is not solely responsible for neuronal damage. The significance of the application resides in the investigation of changes which occur following exposure to hypoxic conditions but prior to irreversible depolarization-dependent glutamate/Ca²⁺ excitotoxic injury. This is indeed a little studied area of ischemic injury. Notwithstanding, the limitations of the application are considerable and significantly diminish the reviewer’s enthusiasm. As noted by the investigator it is not yet established that the very phenomena to be studied, the transient return of evoked synaptic potential, is anything other than an artifact of *in vitro* slice preparations which are already a highly artificial model. The applicability of any findings may be solely limited to the hippocampus and have little general relevance to a more global understanding of ischemia. More importantly, the relevance to *in vivo* ischemic events is suspect and it is difficult to envision how this could be established. Although the investigator states that a long term goal includes the development of neuroprotective strategies, the event being studied (if real) occurs so early in the ischemic process that it may be impossible to develop therapeutic strategies.

Approach: All studies are to be conducted using an *in vitro* hippocampal slice preparation. This is the major weakness of the application. The very elements that the investigator cites as making the slice a superior model to the *in vivo* preparation, are the same considerations which make its utility problematic to understanding *in vivo* responses to ischemic insult. Although the investigator has extensive prior experience in *in vivo* recording techniques there is no proposal to translate *in vitro* observations to *in vivo* conditions. While the preliminary data demonstrate *in vitro* conditions (2 mM Glucose) that allow

separation of the excitatory potential from anoxic depolarization-induced injury, it is not clear what relevance these conditions have to global anoxic phenomena. Only one model of hypoxic-induced neuronal injury is proposed (OGD). Observations may be limited to this singular model as it is known that different metabolic stress models *in vitro* can yield different experimental outcomes. The PI needs to propose some plan to extrapolate data garnered from the current studies to more general phenomenology.

Innovation: This application is somewhat innovative in that it proposes to study ischemia-related events occurring very early in the hypoxia/ischemia cascade. The innovation is diminished since the relevance of the findings may be limited to the experimental model.

Investigators: The PI has been an Associate Professor at the Texas Technical University Health Sciences Center since 1996. Dr. Fowler has a considerable reputation investigating the role of adenosine in synaptic transmission in both normoxic and hypoxic conditions. His productivity in this area has declined considerably; the last noted peer reviewed publication is listed as having appeared in 2003 (suggesting the work was completed some time before that date). Notwithstanding, Dr. Fowler's training, background and experience are more than adequate to complete the proposed investigations.

Environment: The equipment and laboratory facilities listed in the application are suitable to the completion of the project. However, the completion of the studies would require the investigator to purchase, set up, and train upon some pieces of critical equipment that are not currently available in the laboratory.

Overall Evaluation: This application is interesting in that it seeks to study hypoxic/ischemic events which precede the widely studied anoxic depolarization associated with neuronal damage. Enthusiasm was considerably diminished by apparent limitations of any observations to the experimental system proposed for use; it is not clear that the findings will generalize to the overall understanding of hypoxic/ischemic injury. This needs to be more fully justified by the investigator.

CRITIQUE 3:

Overall Evaluation: Stroke is a major cause of morbidity and mortality in the United States. A major component of cognitive deficits after stroke is due to injury involving the hippocampus. The hippocampus is subject to depolarization dependent Ca^{++} /glutamate excitotoxic injury with hypoxia and glucose deprivation. The investigator proposes that ischemic return of evoked synaptic transmission contributes to ischemic injury in hippocampal pyramidal neurons in the CA1. The PI proposes to explore the electrophysiological parameters associated with ischemic return to elucidate novel insights into mechanisms of ischemic injury that may differ substantially from the traditional concepts associated with depolarization-associated glutamate/ Ca neurotoxicity.

The background is generally well written. The pilot data in figures 1-3 suggest that the investigator has developed *in vitro* conditions that separate ischemic return of evoked synaptic transmission from anoxic depolarization in the hippocampal slice. They also show how afferent stimulation impairs recovery of the population spike. Later pilot data is more difficult to follow and the graphs (eg figure 6) are hard to read. Although the investigator provides significant pilot data with this model, as presented, it is difficult to follow how the aims will lead to potential new therapeutic targets for stroke. Further elaboration of the significance of this particular aspect of glutamate/ Ca^{++} excitotoxicity with respect to potential therapeutic treatment strategies would strengthen this application. Additional explanation of previous *in vivo* observations with respect to ischemic return of evoked synaptic transmission may assist in setting the stage for translational potential. Dr. Tryba will be consulting to set-up intracellular recordings. It appears that preliminary data was presented with Dr. Tryba using hippocampal slices. Although the PI has expertise with *in vivo* stroke models, Dr. Tryba has previous expertise with patch recordings in slices. Therefore a more extensive description of this collaboration and the role that Dr. Tryba will have to ensure the success of the study seems justified.

NOTICE: The NIH has modified its policy regarding the receipt of amended applications. Detailed information can be found by accessing the following URL address:
<http://grants.nih.gov/grants/policy/amendedapps.htm>

NIH announced implementation of Modular Research Grants in the December 18, 1998 issue of the NIH Guide to Grants and Contracts. The main feature of this concept is that grant applications (R01, R03, R21, R15) will request direct costs in \$25,000 modules, without budget detail for individual categories. Further information can be obtained from the Modular Grants Web site at <http://grants.nih.gov/grants/funding/modular/modular.htm>

MEETING ROSTER

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ZRG1 BDCN-L (90) S
October 06, 2005 - October 07, 2005

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Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.

NOTIFICATION OF SCIENTIFIC REVIEW ACTION

Release Date: 11/04/2005

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Our Reference: 1 R21 NS055087-01 ZRG1 BDCN-L (90)

The scientific merit review of your application, referenced above, is complete. As part of this initial review, reviewers were asked to provide written evaluations of each application and to identify those with the highest scientific merit, generally the top half of applications they customarily review, for discussion at the meeting and assignment of a priority score. Your application did not receive a score. Unscored applications are neither routinely reviewed at a second level by a national advisory council or board nor considered for funding.

Enclosed is your summary statement containing the reviewers' comments. You should call the program official listed below to discuss your options and obtain advice.

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301-496-1431
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If you choose to resubmit, it is important to respond specifically to comments in the summary statement, as outlined in the instructions in the PHS 398 application kit (<http://grants1.nih.gov/grants/funding/phs398/phs398.html>).

Enclosure

cc: Business or institutional official of applicant organization

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