Impact of the *Xenopus* system on the missions of the NIMH

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Contributions of research in *Xenopus* to the understanding of major psychiatric and neurodegenerative disorders: The *Xenopus* system has led to, and continues to lead to, fundamental advances in understanding the mechanisms of mood stabilizing drugs. Lithium is the most effective and widely used treatment for bipolar disorder, a mood disorder that affects more than 2 million Americans and more than 50 million people worldwide, and yet the mechanism of lithium action remains uncertain. Lithium also disrupts the early development of *Xenopus* embryos, and this robust phenotype has been used extensively to explore the molecular mechanisms of lithium action. One of the most actively investigated mechanisms for lithium action is the inositol depletion hypothesis, and some of the strongest and most frequently cited support for this hypothesis comes from seminal papers using *Xenopus*, including the classic work from Busa and Gimlich, who provided the strongest in vivo data to date showing that exogenous inositol can reverse effects of lithium on phosphatidylinositol signaling. Their findings provide a cornerstone of the inositol depletion hypothesis.

The NIMH also supports research in *Xenopus* that led to the discovery that lithium inhibits the signaling kinase GSK-3 and thereby activates Wnt and neurotrophin/RTK signaling pathways. This discovery provided a compelling alternative mechanism for the developmental effects of lithium in *Xenopus*, directly led to extensive research on the role of GSK-3 in neuronal signaling in mammalian systems, including humans, and led to clinical trials applying GSK-3 inhibitors for neuropsychiatric and neurodegenerative disorders. NIMH funded research in *Xenopus* also led to studies on lithium and GSK-3 in neuronal regeneration, mammalian behavior, Alzheimer's disease, and other neuropsychiatric disease models.

NIMH funded research in *Xenopus* also directly contributed to the discovery that another widely prescribed mood stabilizing and antiepileptic medication, valproic acid, is a direct inhibitor of histone deacetylases (HDACs). These findings are immediately relevant to the mission of the NIMH, but have also had an important impact on research outside the Institute's mandate, including the development of over 40 clinical trials (see http://clinicaltrials.gov/ and search "valproic acid") using valproic acid to treat neurodegenerative, neuromuscular, and neoplastic disorders, and potentially to activate latent HIV in the treatment of AIDS. Inhibition of HDACs also provides a compelling molecular mechanism for the devastating birth defects associated with the use of valproic acid in humans during pregnancy.

Future Directions for the use of *Xenopus* in research on signaling in psychiatric and neurodegenerative disorders: *Xenopus* is an ideal system for future studies on the mechanisms of mood stabilizer drug action, as *Xenopus* embryos and oocytes provide readily accessible, in vivo systems to query the effects of both small molecules and gene products on canonical signaling pathways, including Wnt, TGF-ß/BMP, and FGF pathways, that have been worked out to a great extent in this model system. *Xenopus* oocytes are widely used vehicles for the study of ion channels and G protein coupled receptors that mediate neurotransmitter signaling, and have been one of the classical systems to study cell cycle regulators, posttranscriptional regulation of RNA, and the analysis of small RNA species. *Xenopus* embryos have been, and continue to be, an essential model system for characterizing the molecular mechanisms of Wnt and TGFß signaling. As these pathways are now believed to be important in the pathogenesis of major psychiatric disorders in humans, including schizophrenia and bipolar disorder associated with mutations in the *DISC1* gene, the *Xenopus* system will

remain an important tool to advance our basic understanding of mental illnesses and to translate these basic discoveries to the treatment of psychiatric disorders.

Xenopus as a model for understanding neurodevelopment and behavior: Of high priority to NIMH objectives is the mechanistic understanding of the links between genetics, nervous system structure as established during embryogenesis, and behavior. *Xenopus* is an ideal vertebrate model system for this purpose because it is uniquely amenable to state-of-the-art functional approaches that target every step along the genetics-behavior axis.

Xenopus is a very popular system for neurodevelopmental studies, with a plethora of information available on the molecular genetics of patterning of the CNS and peripheral innervation. It is also very easy to perturb gene function via gain- and loss-of-function approaches (morpholinos, RNAi, dominant negative and mutant construct misexpression). Likewise, many of the antibodies and RNA probes revealing specific components of the sensory and nervous systems are available and work well in Xenopus. Thus, not only are the mechanics of neural structure being unraveled in this system, but any protein of interest (e.g., candidates for human diseases or syndromes) can rapidly and inexpensively be tested. Because the frog embryo can be manipulated from before fertilization, and completes all of its developmental events *in vitro*, it is a model system in which every aspect of nervous system development and behavior can be tracked (and modulated), from the earliest stages of neural induction through to mature animal social behavior.

Moreover, *Xenopus* possesses unique advantages for this work. First, the neurophysiology community routinely tests ion channel, neurotransmitter, and related proteins in the *Xenopus* oocytes assay, which makes a huge toolkit of well-characterized constructs available that have already been tested to a high level of mechanistic detail in this system (Adams et al., 2006; Levin et al., 2002). This also means that not only can biophysical factors (long-term transmembrane voltage gradients etc.) be studied in addition to secreted factors/ECM, but pre-nervous and nervous morphogenetic roles of small molecule neurotransmitters are readily addressed (Levin et al., 2006). Second, unlike in the zebrafish embryo, early *Xenopus* blastomeres have a determined fate-map (Dale and Slack, 1987; Moody, 1987), which means that specific regions of the nervous system can be targeted by microinjection. For example, one can target one side of the brain with a specific mRNA leaving the contralateral side of the animal as an internal control. This is particularly useful for characterization of brain laterality (Wassersug et al., 1999; Wassersug and Yamashita, 2002), a fascinating topic of high relevance to a number of NIMH priority areas.

Most importantly, *Xenopus* is a model system that provides unique opportunities in cognitive science and ethology. *Xenopus laevis* larvae have been a popular behavioral system for investigation of responses to light and gravity, in individual behaviors and schooling (Copp and McKenzie, 1984; Jamieson and Roberts, 2000; Katz et al., 1981; Lum et al., 1982; Moriya et al., 1996; Pronych et al., 1996; Roberts, 1978; Rot-Nikcevic and Wassersug, 2004; Wassersug and Hessler, 1971). Unlike zebrafish and similar model systems, *Xenopus* tadpoles exhibit complex and rich behavioral patterns as larvae, performing schooling and conspecific recognition within 1 week of fertilization. Thus, *Xenopus* tadpoles can be analyzed for behavior, sensory abilities, learning/memory, and social interactions. These are highly sophisticated animals and yet are small enough to be easily amenable high-throughput automated behavioral analysis technology (Hicks et al., 2006). Thus, the effects of neurotoxins, or putative nootropics (drugs that augment memory or learning rate) can easily be characterized in animals that are mutant, wild-type, or modified by mRNA microinjection or pharmacological treatments. Similarly, the molecular basis of memory and learning pathways are readily addressed in *Xenopus*, since the larvae are readily trained at many stages of development and amenable to surgical, pharmacological, and genetic intervention.

Nearly all of the NIMH priority areas can be advanced significantly by segments of the *Xenopus* community, due to this vertebrate model system's combination of accessibility to molecular-genetic, biophysical, and pharmacological approaches and rich behavioral repertoire that will help us with the exciting and biomedically-crucial task of understanding how embryogenesis ultimately gives rise to coherent behavior and cognitive abilities.

Selected references

Adams, D. S., et al., 2006. Early, H+-V-ATPase-dependent proton flux is necessary for consistent left-right patterning of non-mammalian vertebrates. *Development*. 133**,** 1657-1671.

Berridge, M. J., C. P. Downes and M. R. Hanley (1989). "Neural and developmental actions of lithium: a unifying hypothesis." *Cell* **59**: 411-419.

Bosmans F, Martin-Eauclaire MF, Swartz KJ. 2008. Deconstructing voltage sensor function and pharmacology in sodium channels. *Nature* 456:202-208.

Dahan E, Bize V, Lehnert T, Horisberger JD, Gijs MA. 2008. Rapid fluidic exchange microsystem for recording of fast ion channel kinetics in Xenopus oocytes. *Lab Chip* 8:1809-1818.

Dulcis D, Spitzer NC. 2008. Illumination controls differentiation of dopamine neurons regulating behaviour. *Nature* 456:195-201.

Ferrari MC, Chivers DP. 2009. Latent inhibition of predator recognition by embryonic amphibians. *Biol Lett* 5:160-162.

Fraker ME. 2009. The effect of prior experience on a prey's current perceived risk. *Oecologia* 158:765-774.

Fukumoto T, Blakely R, Levin M. 2005a. Serotonin transporter function is an early step in left-right patterning in chick and frog embryos. *Dev Neurosci* 27:349-363.

Fukumoto T, Kema IP, Levin M. 2005b. Serotonin signaling is a very early step in patterning of the left-right axis in chick and frog embryos. *Curr Biol* 15:794-803.

Fuentealba, L. C., E. Eivers, A. Ikeda, C. Hurtado, H. Kuroda, E. M. Pera and E. M. De Robertis (2007). "Integrating patterning signals: Wnt/GSK3 regulates the duration of the BMP/Smad1 signal." *Cell* **131**: 980-93.

Gould, T. D., A. M. Picchini, H. Einat and H. K. Manji (2006). "Targeting glycogen synthase kinase-3 in the CNS: implications for the development of new treatments for mood disorders." *Current Drug Targets* **7**: 1399-409.

Gurvich, N., O. M. Tsygankova, J. L. Meinkoth and P. S. Klein (2004). "Histone deacetylase is a target of valproic acid-mediated cellular differentiation." *Cancer Research* **64**:1079-86.

Hedgepeth, C., L. Conrad, Z. Zhang, H. Huang, V. Lee and P. Klein (1997). "Activation of the Wnt Signaling Pathway: A Molecular Mechanism for Lithium Action." *Dev Biol* **185**: 82-91.

Hicks, C., et al., 2006. Automated analysis of behavior: A computer-controlled system for drug screening and the investigation of learning. *J Neurobiol.* 66**,** 977-90.

Ireland DH, Wirsing AJ, Murray DL. 2007. Phenotypically plastic responses of green frog embryos to conflicting predation risk. *Oecologia* 152:162-168.

Jamieson, D., Roberts, A., 2000. Responses of young Xenopus laevis tadpoles to light dimming: possible roles for the pineal eye. *J Exp Biol*. 203 Pt 12**,** 1857-67.

Klein, P. S. and D. A. Melton (1996). "A molecular mechanism for the effect of lithium on development." *Proc. Nat'l. Acad. Sci. U.S.A.* **93**: 8455-8459.

Levin, M., et al., 2006. Of minds and embryos: left-right asymmetry and the serotonergic controls of pre-neural morphogenesis. *Dev Neurosci*. 28**,** 171-85.

Levin, M., et al., 2002. Asymmetries in H+/K+-ATPase and cell membrane potentials comprise a very early step in left-right patterning. *Cell*. 111**,** 77-89.

Mao, Y., X. Ge, C. L. Frank, J. M. Madison, A. N. Koehler, M. K. Doud, C. Tassa, E. M. Berry, T. Soda, K. K. Singh, T. Biechele, T. L. Petryshen, R. T. Moon, S. J. Haggarty and L. H. Tsai (2009). "Disrupted in schizophrenia 1 regulates neuronal progenitor proliferation via modulation of GSK3beta/beta-catenin signaling." *Cell* **136**(6): 1017-31.

Maslanski, J. A., L. Leshko and W. B. Busa (1992). "Lithium-sensitive production of inositol phosphates during amphibian embryonic mesoderm induction." *Science* **256**: 243-245.

Phiel, C. J., C. A. Wilson, V. M. Lee and P. S. Klein (2003). "GSK-3alpha regulates production of Alzheimer's disease amyloid-beta peptides." *Nature* **423**: 435-9.

Phiel, C. J., F. Zhang, E. Y. Huang, M. G. Guenther, M. A. Lazar and P. S. Klein (2001). "Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen." *Journal of Biological Chemistry* **276**: 36734-41.

Pilcher, H. R. (2003). "Drug research: the ups and downs of lithium." *Nature* **425**: 118-20.

Schloesser, R.J.,J.Huang, P.S. Klein and H.K. Manji (2008)."Cellular plasticity cascades in the pathophysiology and treatment of bipolar disorder." *Neuropsychopharm.* **33**:110-33.

Tsai, L. K., M. S. Tsai, C. H. Ting and H. Li (2008). "Multiple therapeutic effects of valproic acid in spinal muscular atrophy model mice." *Journal of Molecular Medicine* **86**(11): 1243-54.

Yin, L., J. Wang, P. S. Klein and M. A. Lazar (2006). "Nuclear receptor Rev-erbalpha is a critical lithium-sensitive component of the circadian clock." *Science* **311**: 1002-5.

Xenopus Grants funding by the NIMH

According to NIH RePORTER Search Tool, in the fiscal year of 2009, the National Institute of Mental Health (NIMH) **funded 10 grants** for projects involving *Xenopus*. These grants total **$3,564,092**. See appendix for a complete list.

2009 *Xenopus* White Paper – Community Needs

Executive Summary

Xenopus - a crucial model organism for biomedical research:

Experiments in model animals are a cornerstone of biomedical research and have a massive impact on our understanding of human health and disease. The frog, *Xenopus,* is a widely used and crucial vertebrate model organism that offers a unique combination of three powerful advantages: strong conservation of essential biological mechanisms, a remarkable experimental repertoire, and unparalleled cost-effectiveness when compared to murine or other mammalian models.

In fact, for many experimental applications, *Xenopus* is the only viable model system. For example, in cell and molecular biology, *Xenopus* extracts allow for individual components of the cell cycle or DNA replication/repair machinery to be analyzed in a manner that cannot be recapitulated *in vivo* or in cell culture. For developmental biology, no other model system allows for high-throughput genomic/proteomic screening and at the same time allows for transplant/explant analysis (i.e. "experimental embryology"). The *Xenopus* oocyte is unique as a system for studying channel physiology using the patch-clamp and as a system for protein expression. Finally, *Xenopus* is the only vertebrate model that readily produces enough biological material for biochemical purification from eggs, intact embryos, or isolated embryonic tissues. The combination of these characteristics offers a wide range of experimental opportunities for studies using the *Xenopus* system in contrast to other vertebrates such as the mouse or zebrafish.

NIH Investment in *Xenopus:*

The NIH has made a substantial and continuing investment in *Xenopus* research. Indeed, a search of the NIH rePORT database for R01's or equivalent grants using the search term "*Xenopus*" returned **427 grants for a total cost of $127,583,776** for FY08 and FY09. Despite this investment in individuals' research, the *Xenopus* community lacks many resources that are considered entirely essential for other model systems, including a complete genome sequence, stock and training centers, and a comprehensive model organism database.

Xenopus as a Model System and Human Disease:

Given the tremendous advantages of the *Xenopus* system, the pace of new biological discovery by the *Xenopus* Community is brisk. Using *Xenopus*, we have significantly improved our understanding of human disease genes and their mechanisms, justifying the NIH's investment in *Xenopus*. Below we provide examples of just a few of the human health discoveries made in the last two years:

Xenopus embryos are used for *in vivo* analysis of gene expression and function:

Nephronophthisis - *Hum Mol Genet.* 2008. 17, 3655-62; *Nat Genet. 2005*. 37, 537-43.
Cutis laxa - *Nat Genet.* 2009. 41, 1016-21.
Meckel-Gruber syndrome - *Am J Hum Genet.* 2008. 82, 959-70.
Colorectal cancer - *Genome Res.* 2009. 19, 987-93.

Xenopus egg extracts are used for *in vitro* biochemical studies:

Fanconi Anemia - *Mol. Cell.* 2009. 35, 704-15; *J Biol Chem.* 2009, 284, 25560-8.
C-myc oncogene - *Nature*. 2007. 448, 445-51.
BRCA1 - *Cell*. 2006. 127, 539-552

Xenopus oocytes are used to study gene expression and channel activity:

Trypanosome transmission - *Nature* 2009. 459, 213-217.
Epilepsy, ataxia, sensorineural deafness - *N Engl J Med.* 360, 1960-70.
Catastrophic cardiac arrhythmia (Long-QT syndrome) - *PNAS* 2009. 106,13082-7.
Megalencephalic leukoencephalopathy - *Hum Mol Genet.* 2008. 17, 3728-39.

Xenopus as a Model System and Basic Biological Processes:

Xenopus also plays a crucial role in elucidating the basic cellular and biochemical mechanisms underlying the entire spectrum of human pathologies. Again only a few of the many discoveries in the last two years are highlighted here:

Xenopus embryos were used for studies of TGF-® signal transduction.
(*Cell*. 2009. 136,123-35; *Science*. 2007. 315, 840-3).

Xenopus egg extracts revealed fundamental aspects of cell division.
(*Nature*. 2008. 453, 1132-6; *Science*. 2008. 319, 469-72).

Xenopus embryos were used for studying mucociliary epithelia.
(*Nat Genet.* 2008. 40, 871-9; *Nature*. 2007. 447, 97-101).

Xenopus embryos were used for studying development of the vasculature.
(*Cell*. 2008.135, 1053-64).

Xenopus egg extracts provided key insight into DNA damage responses.
(*Mol Cell.* 2009. 35,704-15; *Cell.* 2008. 134, 969-80).

Xenopus embryos linked telomerase to Wnt signaling.
(*Nature*. 2009. 460, 66-72).

Xenopus was used for small molecule screens to develop therapeutics.
(Nat Chem Biol. 2008. 4, 119-25; Blood. 2009. 114, 1110-22).

Immediate Needs of the *Xenopus* Community:

It is the consensus of the *Xenopus* community that their biomedical research could be greatly accelerated by the development of key resources that are currently lacking. These resources would be of use to the entire *Xenopus* research community. In this White Paper, the community identifies seven resources in two categories: Three Immediate Needs and four Essential Resources:

The **Immediate Needs** are a common set of key resources that were identified as the most pressing by three committees established to identify needed resources across the broad and diverse *Xenopus* community. There is a broad, community-wide consensus that these resources would have an immediate impact on all *Xenopus* users and should be assigned the highest priority in order to accelerate the pace of biomedical research using *Xenopus* as a model system.

These Immediate Needs and the resulting improvements in biomedical research are as follows:

1. *Establishment of the Xenopus Resource and Training Center at the MBL in Woods Hole.*
   -Will allow rapid distribution of transgenic *Xenopus laevis* lines expressing fluorescent reporters and tagged proteins (for example histone-RFP for visualizing the mitotic spindle or organ specific GFP in embryos)
   -Will allow centralized generation, housing, and distribution of genetically modified *X. tropicalis* lines, including both mutants and transgenics.
   -Will allow both current investigators and the next generation of researchers to get hands-on training in *Xenopus*-based biomedical research methods (including cell, molecular, and developmental methods).
2. *Expansion and improvement of Xenbase, a Xenopus model organism database.*
   -Maintain and curate data for the essential primary database for *Xenopus* researchers.

-Enhance the functionality of *Xenbase* by introducing a phenotypes feature.
-Support new content on *Xenbase*, including proteomics support, a new genome browser, and Wiki for *Xenopus* methods.
-Continue and expand collaborative and service efforts (e.g. provide *Xenopus* data to other databases including NCBI, UniProtK, Mascot and Tornado).

3. *Complete sequencing of the Xenopus laevis genome.*
-Will allow the deconvolution of data in mass-spectrometry-based proteomic studies.
-Will facilitate identification of conserved gene regulatory regions to build gene-regulatory networks.
-Will facilitate site-specifc studies of DNA transaction (repair and replication)
-Will facilitate identification of all ORFs to build an ORFeome for rapid functional characterization of genes
-Will facilitate the design of morpholino oligonucleotides for gene depletion studies
-Will faciliate the analysis of chromatin-immunoprecipitations to identify DNA-bound to transcription factors and DNA modifications.

Essential Resources Needed by the *Xenopus* Community:

In addition to these immediate, community-wide needs, the committees identified four **Essential Resources** that should be developed as soon as possible, so that *Xenopus* biologists can more effectively fulfill the missions of the NIH. The *Xenopus* community considers all four of these additional resources to be essential, but understands that priorities must be set, and ranks these behind the Immediate Needs. These Essential Resources are as follows:

4. *Xenopus ORFeome in recombineering vectors.*
5. *Improvement of the X. tropicalis genome sequence and annotation*
6. *Development of methods for disrupting gene function in Xenopus.*
7. *Generation and Distribution of antibodies for Xenopus research.*

Anticipated Gains for Biomedical Research:

Xenopus is a crucial model organism for biomedical research. With the development of large-scale community-wide resources, *Xenopus* is poised to be become the premier vertebrate model for systems-level approaches to understanding biological mechanisms in cell, molecular, and developmental biology.

The National Research Council and the National Academy of Sciences have recently called on the Unites States “to launch a new multiagency, multiyear, and multidisciplinary initiative to capitalize on the extraordinary advances recently made in biology”. This report (http://www.nap.edu/catalog.php?record_id=12764) recommends the term "new biology" to describe an approach to research where “physicists, chemists, computer scientists, engineers, mathematicians, and other scientists are integrated into the field of biology.” The promise of systems-level analysis in *Xenopus*, combined with its already proven strengths, make *Xenopus* the ideal model organism for pursuing this “new biology.”

Genome improvements will provide *Xenopus* researchers with the ability to perform genome-wide screens for biological activities that will in turn allow the rapid assembly and analysis of gene regulatory networks. The ORFeome will greatly facilitate such genome-wide screening by allowing all ORFs to be rapidly analyzed or large numbers of proteins to be tagged for analysis of protein-protein interaction or for *in vivo* visualization. Using extracts and biochemical purification coupled with mass-spectrometry and genomic sequence, protein interactomes can be rapidly identified and validated. Because *Xenopus* can be so easily manipulated and because vast amounts of biological material can be generated, cell-type specific interactomes can also be identified. Large-scale genetic screens will identify important novel genes in developmental pathways, especially given the relatively simple genome of *X. tropicalis* compared to zebrafish. Finally, the flexibility of both *Xenopus* extracts and embryos make this system ideal for chemical biology screens. Identifying these gene-regulatory networks, interactomes, and novel genes will be only the first steps, of course. The well-

established power of *Xenopus* for rapid analysis of gene function will then allow deeply mechanistic analyses to complement the systems-level approaches described above.

It is the combination of these characteristics that distinguishes *Xenopus* from other vertebrate model systems such as mouse and zebrafish and allows for a systems-level approach to understanding biological mechanisms. The tremendous promise of the *Xenopus* model cannot be realized, however, without the immediate development of community-wide research resources. This White Paper presents the needed resources, and we look to the NIH for guidance in how to best achieve these goals.

For complete details of the 2009 Xenopus White Paper, please visit
http://www.xenbase.org/community/xenopuswhitepaper.do

Appendix

Xenopus Grants funded by the NIMH

Project Number	Activity	Project Title	Principal Investigator	Organization	Total
1R03MH086789-01	R03	KV4.3 GATING CURRENT: MECHANISMS UNDERLYING CLOSED STATE INACTIVATION	CAMPBELL, DONALD LEROY	STATE UNIVERSITY OF NEW YORK AT BUFFALO	$77,439
5R01MH059937-10	R01	MOLECULAR PHYSIOLOGY OF MAMMALIAN INSP3 RECEPTOR	FOSKETT, J. KEVIN	UNIVERSITY OF PENNSYLVANIA	$300,573
1P50MH086383-01	P50	NICOTINIC CHOLINERGIC RECEPTOR AGONISTS	FREEDMAN, ROBERT R	UNIVERSITY OF COLORADO DENVER	$130,564
5R37MH065334-29	R37	GENETIC STUDIES OF THE SYNAPSE	JAN, LILY Y	UNIVERSITY OF CALIFORNIA SAN FRANCISCO	$375,040
1U19MH085193-01A1	U19	DESIGN AND STUDY OF NEW NICOTINIC ANALOGS FOR USE IN DEPRESSION	KOZIKOWSKI, ALAN PAUL	UNIVERSITY OF ILLINOIS AT CHICAGO	$964,233
1R01MH081842-01A2	R01	AUTOMATED ANALYSIS OF LEARNING AND MEMORY FOR NEURO-DEVELOPMENTAL STUDIES	LEVIN, MICHAEL	TUFTS UNIVERSITY MEDFORD	$378,513
1R01MH079381-01A2	R01	TOL2-MEDIATED GENE AND ENHANCER TRAPPING IN XENOPUS TROPICALIS	MEAD, PAUL E	ST. JUDE CHILDREN'S RESEARCH HOSPITAL	$414,665
5R01MH060252-07	R01	CLEFT BINDING NMDA RECEPTOR SUBTYPE ANTAGONISTS	MONAGHAN, DANIEL T	UNIVERSITY OF NEBRASKA MEDICAL CENTER	$269,874
5R01MH074702-05	R01	MECHANISMS OF CALCIUM TRANSIENTS IN NEURONAL DEVELOPMENT	SPITZER, NICHOLAS C	UNIVERSITY OF CALIFORNIA SAN DIEGO	$316,150

5R01MH071404-04	R01	NETRIN SIGNALING DURING NEURONAL DEVELOPMENT	STEIN, ELKE	YALE UNIVERSITY	$337,032
				Total	$3,564,092