Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2010 - 12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814 935 1081

5. **Grant SAP Number:** 4100050904

6. **Project Number and Title of Research Project:** 32. Epigenetic Changes in Gene Expression Associated with an Anxious Depressive like Phenotype

7. **Start and End Date of Research Project:** 9/1/2010 - 6/30/2012

8. **Name of Principal Investigator for the Research Project:** Bernhard Lüscher, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 58,960

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Thomas Fuchs	Postdoc	100% (2)	$22,772.29
Qiuying Shen	Graduate student/postdoc	100% (2)	$6,128.72
Zhen Ren	Graduate student	50% (1)	$10,848.60

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
Bernhard Luscher	Professor of Biology and Biochemistry and Molecular Biology	5 %
Nadia Sahir	Postdoc	15%

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No___x___

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes___x____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If

you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
A novel developmental mouse model of major depressive disorder	X NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:___)	Nov 2011	$ 409,751	$ 409,751
GABAergic control of depression-related brain states	X NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:__)	July 2012	$ 2,717,969	$ 2,466,355

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes___x____ No_____

If yes, please describe your plans:

We are working on an NIH R21 grant proposal to further pursue investigation of a couple of the candidate genes that are differentially expressed in in $\gamma2^{+/-}$ vs. WT mice.

12. Future of Research Project. What are the future plans for this research project?

We plan to use the $\gamma2^{+/-}$ model of depression to gain further insights into the etiopathology of major depression. The $\gamma2^{+/-}$ model has unique construct and face validity for a mouse model of major depressive disorder with respect to behavioral (behavioral changes that are the inverse of those induced by antidepressants), cognitive (deficits in the resolution of ambiguities, pattern formation), neuroendocrine (increased serum corticosterone), pharmacological (increased sensitivity to antidepressant drugs), as well as cellular phenotypes (i.e. defects in hippocampal neurogenesis). Unlike chronic stress based models pursued by most other labs, our model represents an endogenous model that may model the increased vulnerability to major depression rather than a depressive state alone. First, we are working on determining the developmental origin of this phenotype. Second, recent evidence

from our lab suggests that the GABAAR deficit in these mice results in significant deficits in glutamatergic transmission that can be rescued by antidepressant drug treatment. These preliminary experiments further validate the value of this model and suggest that GABAergic maybe causal for glutamatergic deficits that have been independently implicated in the etiopathology of major depressive disorder.

13. **New Investigator Training and Development**. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes___x___ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male			1	1
Female			1	
Unknown				
Total			**2**	**1**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			2	1
Unknown				
Total			**2**	**1**

	Undergraduate	Masters	Pre-doc	Post-doc
White			1	1
Black				
Asian			1	
Other				
Unknown				
Total			**2**	**1**

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No___X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes___X___ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

The project enabled the acquisition of preliminary data that were essential for the successful competition for NIH grants.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No___X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No___X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X___

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.
List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the

project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Goal of experiments

The goal of our project was to take advantage of a genetically defined animal model of anxious depression (GABA-A receptor gamma2 subunit heterozygous ($\gamma 2^{+/-}$) mice to identify gene expression changes and molecular pathways involved in the etiology of depression. Based on our published behavioral pharmacological studies we had predicted that $\gamma 2^{+/-}$ mice exhibit chromatin modifications and gene expression changes in the hippocampus and/or frontal cortex that are normalized by chronic treatment with the tricyclic antidepressant desipramine but not with the selective serotonin reuptake inhibitor (SSRI) fluoxetine. As a measure of gene expression we had proposed to use ChIPSeq analyses and to validate putative differences in gene expression by QPCR of cDNA prepared from the same brain areas of mice.

Summary:

In retrospect the goal of our proposal was too ambitious for a pilot project – the funds and time allocated to our project was insufficient to thoroughly pursue our ideas. First, we underestimated the challenges of chromatin immunoprecipitation from small amounts of brain tissue. After our initial attempts to get gene expression data through a ChIPSeq approach had failed we realized that time and funds were insufficient to further pursue this strategy. Instead we continued previously initiated exon microarray analyses to get information on gene expression changes. Although these experiments looked promising initially, thorough statistical analyses and attempts

to verify candidate gene expression changes by RTPCR eventually revealed that the effect sizes of gene expression changes were likely smaller than predicted, while individual variation in gene expression that were unrelated to genetic manipulation of our model seemed to occlude the gene expression changes we were looking for. In retrospect, we learned from these experiments that the effect sizes of gene expression changes that may be observed in our mouse model requires larger group sizes of animals. In the future deep sequencing or microarrays will be preferable over ChIPSeq as corresponding measures are less subject to technical variation and therefore better suited to discover small effects. Other emerging technologies such as Nanostring nCounter (Nanostring) analyses of candidate genes, which directly count transcripts and do not require amplification of transcripts, may be even more promising for identification of small yet biologically relevant changes in gene expression.

Detailed progress report

Production of mice and tissue harvest:

The mice to be used for experiments (6 females each per genotype (WT and $\gamma2^{+/-}$) and treatment (fluoxetine, desipramine, vehicle) were produced by crossing WT and $\gamma2^{+/-}$ mice. The offspring females were genotyped at 3 weeks of age and housed two mice per cage in a regular cage. Starting at eight weeks of age they were treated with fluoxetine or desipramine or vehicle alone (drinking water) for 4 weeks as described (Shen et al. 2010). Mice were decapitated in the room next to the husbandry room to avoid stress and other order of sampling effects (1:30 PM to 4 PM, mice kept on a standard 7AM:7PM light cycle) and the brains quickly dissected by hand using a Brain Matrix and Blades (Electron Microscopy Sciences). Four consecutive 2 mm sections from Bregma + 2.4 to 5.6 (Paxinos and Franklin, 2001) were transferred to ice cold phosphate buffered saline (PBS). The cingulate cortex (2-3 punches, Gauge 15, per animal, in total) was punched from the first two sections and included part of the prelimbic cortex. The nucleus accumbens (2 punches) was obtained from the second section. The amygdala (2 punches) was obtained from the third section and the hippocampus (4 punches) from the third and fourth sections. The punches from two animals treated identically were combined and suspended in pre-labeled tubes containing 940 µL of 1% formaldehyde in PBS at room temperature for 12 minutes. Crosslinking was stopped by addition of 64 µL of 2M glycine. The tissue was washed with PBS and stored at -80 degree C for use once the protocols were optimized.

Adaptation of chromatin immunoprecipitation (ChIP) to brain tissue:

We had found in preliminary experiments that fragmenting chromatin from brain tissue by sonication was much more inefficient than fragmenting chromatin from cultured cells. Although we used a tip sonicator instead of a cup sonicator and increased the duration of sonication more than tenfold, this aspect of the ChIP protocol remained suboptimal, producing lower yield than desirable. A total of 20 cycles of 10 sec sonication at setting 2.5 100% output were needed to yield DNA in the 100 – 200-bp range, but the range of size distribution remained larger than we had hoped and this ultimately may have contributed to the problems we faced with deep sequencing (see below). Nevertheless, our ChIP assays seemed to work. PCR amplification of DNA fragments in anti-H3K9me3 chromatin immunoprecipitates with primer pairs specific for MAPK13 and Foxe3 (two genes widely expressed in brain) reliably showed the presence of DNA in the immunoprecipitated samples but not in antibody-lacking negative controls.

ChIPSeq:

After optimizing our ChIP protocol we performed a pilot ChIPSeq experiment from a pair of WT and $GABA_A$ receptor $\gamma2^{+/-}$ test brain samples using an H3K9me3 antibody (ab8898, ABCAM, Cambridge, MA) to capture gene control regions of transcriptionally repressed chromatin. Briefly, DNA fragments immunoprecipitated with anti-H3K9me3 from six (Gauge15) 1-mm tissue punches were phosphorylated with T4 polynucleotide kinase, subject to A-tailing of 3' ends with Klenow Fragment, ligated with sequencing adaptors using T4 DNA ligase, ligated with iLumina 1-16 multiplex bar code adaptor oligonucleotides (different adaptors were used for WT and $\gamma2^{+/-}$ samples). The WT and $\gamma2^{+/-}$ samples were then pooled, the DNA purified by adhesion to magnetic AMPure beads and then subject to size selection by running the sample over a non-denaturing 6% polyacrylamide tris-borate gel along with a DNA size ladder. The gel was briefly stained with ethidium bromide, the 200-300-bp size range of the gel was excised, the gel material was crushed and the DNA fragments were eluted from the gel and subject to limited amplification by PCR (9 cycles). This DNA library was subjected to sequencing using the iLumina sequencing platform, along with reference samples prepared by other investigators from cultured yeast and mammalian cells. We found that the yield in uniquely mappable sequence tags obtained from our sample was > 400 fold lower than expected and far too low to provide a measure of expression of even moderately expressed genes. This low yield was explained in part by an about 5-fold lower amount of total sequences obtained and an about 80-fold lower relative yield in uniquely mappable sequence tags. The experiment indicated that the amount of tissue needed for efficient ChIP, DNA amplification and isolation for sequencing ideally is larger than in our pilot experiment and that the protocol for isolation of DNA would have to be optimized to become orders of magnitude more efficient. It is also likely that some impurities in the DNA had interfered with linker modification and amplification of our samples.

Microarray as alternative strategy:

Given our difficulties with the ChIPSeq approach and the limited amount of time and funds remaining as part of this seed grant we decided to change strategy and instead of ChIPSeq to extend our exploration of microarray analyses as a means to elucidate gene expression changes. We had isolated RNA from cingulate cortex of a total of 20 mice (5 $\gamma2^{+/-}$ females, 5 WT females, 5 $\gamma2^{+/-}$ males and 5 WT males) and subjected them to analyses by Affymetrix® GeneChip® Mouse Exon 1.0 ST Arrays. All but two of these samples gave reasonable signal intensities suitable for further analyses. The two samples that gave low hybridization signals were discarded. The raw signal intensities of the remaining 18 microarrays were summarized into exon level and gene level expression data, respectively using Affymetrix® Expression Console™ software and normalized using the Robust Multi-array Average algorithm (RMA) (Irizarry et al., 2003, *Biostatistics*, 4, 249-264).

After learning that there is currently no consensus as to what represents the best method to translate raw data from exon microarrays into gene level expression data we decided to compare three different methods to retrieve candidate genes for differential expression in $\gamma2^{+/-}$ vs. WT mice. A first analysis was based on the gene level expression data mentioned above provided by Expression Console (proprietary software provided by Affimetrix). These data were also used to estimate effect sizes (fold changes) of genotype-dependent gene expression changes. In a second approach, the calculated mean values of exon level data provided by Expression Console for each array were used as a value representative of the expression of each gene and sample. In a

third analysis (adopted from Surget et al. *Neuropsychopharmacology*, **34**, 1363–1380), the exon level data were individually compared across arrays and the exon of each gene with the largest genotype difference was then used as representative for the expression of that gene. This last method is specifically designed to maximize sensitivity of detection of specific splice forms of transcripts that are differentially expressed. However, it is also prone to detection of false positives.

Differential expression analysis ($\gamma2^{+/-}$ vs. WT mice) of the gene-specific data obtained by each of the above three methods were then processed using the Limma package (Smyth, 2004, *Statistical Applications in Genetics and Molecular Biology*, Vol. 3, No. 1, Article 3) in R/Bioconductor, either separately for males or females or for the two sexes together. The top 500 genes ranked based on log-odds ratios of each of the nine analyses [three methods x three groups (males, females, both sexes)] were then selected and the P values provided by the Limma package were recorded. To extract the most promising candidates we restricted further analyses to the 144 genes that were represented among the top 500 differentially expressed genes in each of the three analyses and that had uncorrected P values of < 0.05 and a predicted fold change of expression of < 0.67 or > 1.5 in either male, or female mice or both. Among these genes the following seven genes were further pursued for verification, because their known or predicted function was consistent with a role in neuropsychiatric disorders: Sfrp5, Mapk14, Frat2, Carshp1, Tnfsf18, Cripto1, and Tcf4.

<u>Attempts to confirm gene expression changes by RTPCR.</u>
To independently assess gene expression changes of candidate genes by RTPCR, we isolated new RNA from hippocampus and cingulate cortex from ultimately three sequentially bred cohorts of WT and $\gamma2^{+/-}$ littermates (n= 3-5 per sex and genotype). The hippocampus was included in these tests as it yielded significantly larger amounts of RNA per animal, and as an additional reference. RNA was isolated individually from each mouse, transcribed into cDNA and amplified using TaqMan Real Time PCR and custom designed PCR primer pairs (TaqMan,Applied Biosystems) specific for Sfrp5, Mapk14, Frat2, Carshp1, Tnfsf18, Cripto1, and Tcf4 and an ABI 7300 real time PCR machine. In addition, all the samples were also analyzed with primers specific for either GAPD or cyclophilin or both, as internal standards. Duplicate reactions were run from all RNA samples. ΔCT values of individual biological replicates were normalized against values for GAPD and/or cyclophilin, subjected to statistical analyses and used to calculate linear fold-changes ($\Delta\Delta$CT) as described by Schmittgen et al (Nat. Protoc. **3**, 1101-1108). The expression of each of these genes was analyzed in at least two cohorts of WT and $\gamma2^{+/-}$ littermates. Some of the primer sets initially showed significant changes in $\gamma2^{+/-}$ vs. WT brain (effect sizes, 2- 3 fold) but the changes did not match those predicted by miocroarrays. Worse, none of the changes could be reproduced in a second cohort of mice, leading us to conclude that these genes were not reliably affected.

In sum, we concluded that neither the ChIPSeq nor the microarray approaches were successful in elucidating gene expression changes associated with depression-related behavior of the $\gamma2^{+/-}$ mouse model. Future attempts will need to rely on larger cohorts of animals and focus on fewer comparison groups to maximize statistical power of these experiments. Moreover, a candidate gene approach combined with a method that directly counts RNA molecules such as the Nanostring nCounter method maybe preferable over genome wide approaches. This latter method has the advantage that it allows direct counting of transcripts with minimal enzymatic

modification and without amplification, which improves statistical power of analyses.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___x__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic

copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. The GABAergic deficit hypothesis of major depressive disorder.	B. Luscher Q. Shen N. Sahir	Mol. Psychiatry, 16, 383–406	June 2010	☐Submitted ☐Accepted ☑ Published
2. $GABA_A$ receptor trafficking-mediated plasticity of inhibitory synapses	B. Luscher, T. Fuchs C. Kilpatrick	Neuron 12, 385-409	Jan 2011	☐Submitted ☐Accepted ☑ Published
3. GABAergic control of critical developmental periods for anxiety- and depression-related behavior in mice	Q. Shen T. Fuchs N. Sahir B. Luscher	PloS ONE, e47441	June 2012	☐Submitted ☐Accepted ☑ Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes_____ No____X___

If yes, please describe your plans:

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___x

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___

If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No x

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No__X

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No___X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

Bernhard Lüscher

- Professor of Biology and
- Professor of Biochemistry and Molecular Biology

The primary goal of Dr. Lüscher's research is to elucidate the role of GABAergic deficits in the etiology of affective disorders, which we pursue by combining pharmacologic and genetic manipulation of mice with biochemical, cell biological, pharmacological, genomic and behavioral analyses.

He is formally trained as a biochemist and molecular biologist with 9 years of additional postdoctoral training at the Institute of Pharmacology, University of Zurich. He has been studying $GABA_A$Rs and their regulation in vitro and in vivo for 23 years, first in Zurich and for the last 14+ years at Penn State University. In particular, his laboratory has established $GABA_A$R γ2 subunit heterozygous mice as a genetically defined animal model with excellent construct, face and predictive validity for melancholic major depression. This model suggests that deficits in GABAergic transmission via $GABA_A$Rs may be causal for major depressive disorder. Other significant contributions include the demonstration that the $GABA_A$R γ2 subunit and gephyrin are interdependently required for localization and function of $GABA_A$Rs at synapses, the demonstration that postsynaptic $GABA_A$Rs are palmitoylated and that this modification is required for their normal accumulation at synapses as well as for normal synapse formation, the isolation of GODZ/zDHHC3 as a founding member of mammalian family of zDHHC-type palmitoyltransferases, and the isolation of CAML as a $GABA_A$R interacting protein essential for normal endocytic recycling of $GABA_A$Rs.

At Penn State he serves as director of CMIND, an interdepartmental neuroscience center that provides space for 12 neuroscience research groups affiliated with the Biochemistry & Molecular Biology and Biology Departments. Over the years he has trained 19 graduate students and 6 postdoctoral coworkers as well as numerous undergraduate students. He teaches an average of 70 lectures per year, including a core course in Molecular and Cellular Neuroscience (Biol469) for approx. 150 students.

Positions and Employment

1987–1990	Postdoctoral Fellow, University of California, Berkeley, CA (Dr. Robert Tjian)
1990–1998	Oberassistant (Assist. Prof. equivalent), Institute of Pharmacology, University of Zurich
1999–2006	Associate Professor, Dept. of Biology and Dept. of Biochemistry & Molecular Biology, Eberly College of Science, Pennsyania State University (tenured in 2002)
2006–*present*	Professor, Dept. of Biology and Dept. of Biochemistry & Molecular Biology, Eberly College of Science, Dept. of Psychiatry, College of Medicine, Pennsylvania State University

Secondary Appointments and Other Experience

2003–2005	Co-Director Graduate Degree Program in Neuroscience, Penn State University
2004–2008	Interim Co-Director, Penn State Neuroscience Institute, Penn State University
2004–*present*	Director Center for Molecular and Cellular Neuroscience (CMIND), Penn State University

Professional Service

Scientific organizations: 1993–*present* Member Society for Neuroscience, 1997–*present*, Member International Brain Research Organization (IBRO); 2012–*present* Member American Society for Biochemistry and Molecular Biology,

Grant review panels: 2005–2006 Ad hoc member SYN Scientific Review Group; 2005–*present* Ad hoc member, NTRC Scientific Review Group; 2007–2008 Member Special Emphasis Panel NIH BDCN A90/PMDA; 2009 Member Special Emphasis Panels NIH ZMH1 CNF-Z (02) S, ZRG1 BDCN A96S; 2008–*present* Review Editor, Frontiers in Molecular Neuroscience; 2009–2012 Chartered member NIH PMDA Scientific Review Group; 2013 Member NIMH EUREKA panel 2013 ZMH1 ERB-M (07).

Editorial responsibilities: 2008 – present Review Editor, Frontiers in Molecular Neuroscience, 2010 – 2012 'Subunit Chair' of the $GABA_A$ receptor subcommittee on Receptor Nomenclature and Drug Classification of the International Union of Basic and Clinical Pharmacology (NC-IUPHAR), 2012 – 2017 Editorial Board Member, Journal of Biological Chemistry (5-year 3 months term); Ad hoc Reviewer for >35 Journals including: Biol. Psychiatry, Genes, Brain & Behav., J. Biol Chem., J. Cell Sci., J. Neurosci., J. Physiol., J. Neurophysiol., Learn. & Memory, Mol. Neurobiol., Mol. Pharmacol., Mol. Psychiatry, Neurochemistry, Neuron, Nat Neurosci., Nat. Rev. Neurosci., Nat. Protoc., Pharmacol. & Therapeut., Proc. Natl. Acad. Sci. USA, Trends Neurosci., etc.

Key Publications (out of >60)

1. Song, J, C. Zhong, M. Bonaguidi, D. Hsu, H. Davoudi, Y. Gu, K. Meletis, S. Ge, G. Enikolopov, K. Deisseroth, **B. Luscher**, K. Christian, G.-l. Ming and H, Song (2012) Neuronal circuitry mechanism regulating adult quiescent neural stem cell fate decision. Nature, 2012/07/29/online, NIHMSID # 384645
2. Wu, X., Z. Wu, G. Ning, Y. Guo, R. Ali, R.L. Macdonald, A.L. De Blas, **B Luscher**, and G Chen (2012). GABAA receptor alpha subunits play a direct role in synaptic versus extrasynaptic targeting. J. Biol. Chem. [Epub ahead of print] NIHMSID # 394966
3. **Luscher, B**, Q. Shen and N. Sahir (2011) The GABAergic deficit hypothesis of major depressive disorder. Mol Psychiatry 16:383-406. PMCID: PMC3412149
4. **Luscher, B.,** Fuchs, T., Kilpatrick, C. L., 2011. GABA(A) receptor trafficking-mediated plasticity of inhibitory synapses. Neuron 70, 385-409. PMCID: PMC3093971
5. Shen, Q., R. Lal, B. A. Luellen, J.C. Earnheart, A. M. Andrews and **B. Luscher** (2010). GABA(A) receptor deficits cause hypothalamic-pituitary-adrenal axis hyperactivity and antidepressant drug sensitivity characteristic of melancholic depression. Biol Psychiatry 68: 512–520. PMCID: PMC2930197
6. Yuan X., J. Yao, J.S. Qi, D. Norris, D.D. Tran, R.J. Bram, G. Chen and **B. Luscher** (2008) Calcium-Modulating cyclophilin Ligand regulates membrane trafficking of postsynaptic $GABA_A$ receptors. Mol. Cell. Neurosci. 38, 277-289. PMCID: PMC2350232
7. Earnheart JC., C. Schweizer, F. Crestani, T. Iwasato, S. Itohara, H. Mohler, **B. Luscher**. (2007). GABAergic control of adult hippocampal neurogenesis in relation to behavior indicative of chronic trait anxiety and depression states. J. Neurosci. 27, 3845-3854. PMCID: PMC2441879
8. Fang C., L. Deng, C.A. Keller, M. Fukata, Y. Fukata, G. Chen, **B. Luscher** (2006). GODZ-mediated palmitoylation of $GABA_A$ receptors is required for normal assembly and function of GABAergic inhibitory synapses. J. Neurosci. 26, 12758-68. PMCID: PMC2366897
9. Schweizer C., S. Balsiger, H. Bluethmann, I.M. Mansuy, J-M. Fritschy, H. Mohler, and **B. Luscher** (2003). The γ2 subunit of $GABA_A$ receptors is required for maintenance of receptors at mature synapses. Mol. Cell. Neurosci. 24, 442-450.
10. Crestani F., M. Lorez, K. Baer, C. Essrich, D. Benke, J. P. Laurent, C. Belzung, J. M. Fritschy, **B. Luscher**, and H. Mohler (1999). Decreased GABA(A) receptor clustering results in enhanced anxiety and a bias for threat cues. Nat Neurosci 2, 833-839. PMID: 10461223 [Commentaries: McNaughton, N. A. (1999). Nat Med 5, 1131-1132; Anagnostaras et al. (1999). Nat Neurosci **2**, 780-782.
11. Essrich C., M. Lorez, J. Benson, J.-M. Fritschy and **B. Luscher** (1998). Postsynaptic clustering of major $GABA_A$ receptor subtypes requires the γ2 subunit and gephyrin. Nat Neurosci 1, 563-571. [Cover issue, Commentary: Betz, H. Nat. Neurosci. 1, 541-543 (1998)].
12. Günther U., J. Benson, D. Benke, J.-M. Fritschy, G. Reyes, F. Knoflach, F. Crestani, A. Aguzzi, M. Arigoni, Y. Lang, H. Bluethmann, H. Mohler, and **B. Luscher** (1995). Benzodiazepine-insensitive mice generated by targeted disruption of the γ2 subunit gene of GABA(A) receptors. Proc Natl Acad Sci USA 92, 7749-7753.