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:** Temple University – Of The Commonwealth System of Higher Education

2. **Reporting Period (start and end date of grant award period):** 1/12010-12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Germaine A Calicat, MA

4. **Grant Contact Person's Telephone Number:** (215)204.**7**655

5. **Grant SAP Number:** 4100050909

6. **Project Number and Title of Research Project:** Project 12: Are There Sex Differences in Kappa Opioid Receptor-Mediated Pharmacology?

7. **Start and End Date of Research Project:** 12/1/10 – 6/30/11

8. **Name of Principal Investigator for the Research Project:** Lee-Yuan Liu-Chen, 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:

   $ 56,081.20

   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, First Name	Position Title	% of Effort on Project	Cost
Wang	Postdoctoral fellow	100% (supported by the grant 60%)	**$30,000**
Liu-Chen	Professor	10%	$0

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, First Name	Position Title	% of Effort on Project
Rasakham	Postdoctoral fellow	100%
Huang	Associate Scientist	25%
Chudnovskaya	Undergraduate Student	25%
Cowan	Professor	10%
Liu-Chen	Professor	10%

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___x_____ No____ _____

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

NIH grant DA17302 $190,000/year
NIH grant T32DA007237 (fellowship support for Rasakham)

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:
Sex differences in kappa opioid pharmacology	xNIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)	Feb 1, 2011	$275,000/year for 3 years	Not funded
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$

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

Yes_____ No____x_____

If yes, please describe your plans:

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

None

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				
Female	1			2
Unknown				
Total	**1**			**2**

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

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

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.

New investigators were recruited.

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 agreement). Summarize the progress made in achieving these goals, objectives and aims <u>for the period that the project was funded (i.e., from project start date through end date).</u> 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.

Specific Aims

Specific Aim 1. Determine if there is a sex difference in KOPR distribution in the brain
Hypothesis: Sex effects on KOPR distribution occur in a brain-region specific manner
Experiment 1: Determine the affinity and total number of binding sites of [^{3}H]U69,593 binding to KOPR in male and female guinea pigs in phases of the estrous cycle associated with high and low levels of estrogen (proestrus and diestrus, respectively) using whole brain membranes.
Experiment 2: Examine the distribution and expression of KOPR in the brains of male, proestrus female and diestrus female guinea pigs by [^{3}H]U69,593 receptor autoradiography.

Progress made on aim 1: Specific aim 1 was fully achieved. Please see page 8 in this report.

Specific Aim 2. Determine if there is a sex difference KOPR coupling to G-protein in the brain
Hypothesis: Sex effects on KOPR coupling to G-protein occur in a brain-region specific manner
Experiment 1: Examine the efficacy and potency of the KOPR agonist U50,488H in activating G-proteins by [^{35}S]GTPγS binding using whole brain membranes from male, proestrus female and diestrus female guinea pigs.
Experiment 2: Examine U50,488H-induced activation of G-proteins by [^{35}S]GTPγS binding autoradiography in the brains of male, proestrus female and diestrus female guinea pigs.

Progress made on aim 2: Specific aim 2 was fully achieved. Please see page 9 in this report.

Specific Aim 3. Investigate whether there is a sex difference in KOPR agonist-induced modulation of neuronal circuitry in the brain
Hypothesis: *Activation or inhibition of neuronal activity by the KOPR agonist U50,488H is dependent on sex and brain region*
Experiment: Examine U50,488H-induced activation or inhibition of phosphorylation of p44/42 MAPK by immunohistochemistry on brains of male, proestrus female and diestrus female guinea pigs.

Progress made on aim 3: Specific aim 3 was fully achieved. Please see page 9 in this report.

Abstract We have previously shown that sex differences in kappa opioid receptor (KOPR) pharmacology exists in guinea pigs, which are more similar to humans in the expression level and distribution of KOPR in the brain than rats and mice. Males showed greater KOPR-mediated abnormal postures, immobility and antinociception in the paw pressure test than females. In contrast, inhibition of cocaine-induced hyperactivity by U50,488H was more effective in females than males. In this study, we examined if sex differences in KOPR level and KOPR-mediated G protein activation in brain regions may contribute to the observed differences using quantitative *in vitro* autoradiography of [^{3}H]U69,593 binding to the KOPR and U50,488H-stimulated [^{35}S]GTPγS binding. Compared to females, males exhibited greater [^{3}H]U69,593 binding in the deep layers of somatosensory and insular cortices, claustrum, endopiriform nucleus, periaqueductal gray, and substantial nigra. Concomitantly, U50,488H-stimulated [^{35}S]GTPγS binding was greater in males than females in the superficial and deep layers of somatosensory and insular cortices, caudate putamen, claustrum, medial geniculate nucleus and cerebellum. In contrast, compared to males, females showed greater U50,488H-stimulated [^{35}S]GTPγS binding in the dentate gyrus, and a trend of higher [^{35}S]GTPγS binding in the hypothalamus. These data demonstrate that males and females differ in KOPR expression and KOPR-mediated G-protein activation in distinct brain regions, which may contribute to the observed sex differences in KOPR-mediated pharmacology.

Sex Differences in [^{3}H]U69,593 binding to the KOPR

Specific Aim 1 is addressed with the work described here. We compared the levels and distribution of [^{3}H]U69,593 binding to KOPR between male and female guinea pig brains. Representative pseudo-color autoradiograms are shown in Fig. 1. In general, male and female guinea pigs displayed similar regional distribution (Fig. 1). Quantitation of specific [^{3}H]U69,593 binding was obtained by measuring the density of autoradiograms in regions of interest. Table 1 shows the analysis of specific [^{3}H]U69,593 binding. Males exhibit significantly higher [^{3}H]U69,593 binding than females in the following brain regions: deep layers of somatosensory cortex and insular cortex, claustrum, endopiriform nucleus, periaqueductal gray, and substantial nigra. However, in any other brain regions we did not observe any significant sex difference in [^{3}H]U69,593 binding.

Sex Differences in U50,488H-stimulated [^{35}S]GTPγS binding

Specific Aim 2 is addressed with the work described here. U50,488H-stimulated [^{35}S]GTPγS binding was compared between male and female guinea pig brains. Representative pseudo-color autoradiograms of U50,488H-stimulated [^{35}S]GTPγS binding are shown in Fig. 2. Males and females displayed similar neuroanatomical distribution of U50,488H-stimulated [^{35}S]GTPγS binding. Table 2 shows the analysis of basal and net U50,488H-stimulated [^{35}S]GTPγS binding. There is no difference between males and females in basal levels of binding, but there are large regional differences. U50,488H-stimulated [^{35}S]GTPγS binding was significantly greater in males than females in several brain regions, which include superficial and deep layers of somatosensory cortex and insular cortex, caudate putamen, claustrum, medial geniculate nucleus and cerebellum. In contrast, in the dentate gyrus of the hippocampus, females exhibited significantly higher U50,488H-stimulated [^{35}S]GTPγS binding than males. In addition, females exhibited a trend of higher levels in the hypothalamus, but it did not reach statistical significance ($P = 0.09$).

Sex Differences in U50,488H-induced Phosphorylation of p44/42 MAP kinase in the Guinea Pig Brain

Specific Aim 3 is addressed with the work described here.

In the present study we examined if sex differences exist in neural activity induced by the KOPR agonist U50,488H. Here, we used immunohistochemistry to detect activation (phosphorylation) of p44/42 MAPK as an indicator of neural activity. Following habituation to injection for 3 days, adult guinea pigs received a single injection of U50,488H (5 mg/kg, s.c.) and perfused thirty to forty-five minutes later. U50,488H-induced an increase in the number of cells immuno-positive for phosphorylated p44/42 MAPK in subregions of the amygdala, thalamus, paraventricular nucleus of the hypothalamus, periaqueductal grey, and dorsal raphe nuclei. In contrast, U50,488H-induced a decrease in immuno-positive cells in the ventrolateral and lateral orbital cortex. Pretreatment with the KOPR antagonist norBNI (10 mg/kg, i.p.) 18 hrs prior to U50,488H significantly reversed the effects of U50,488H in most regions. In addition, we observed a notable sex difference in the basolateral amygdala; in males, U50, 488H-induced an increase in immuno-positive cell numbers but a decrease in females (Table 3). However, across other brain regions males were generally more sensitive to U50,488H-induced alterations than females (Table 3). These results suggest the need to include female subjects in studies examining emotional responses to KOPR ligands.

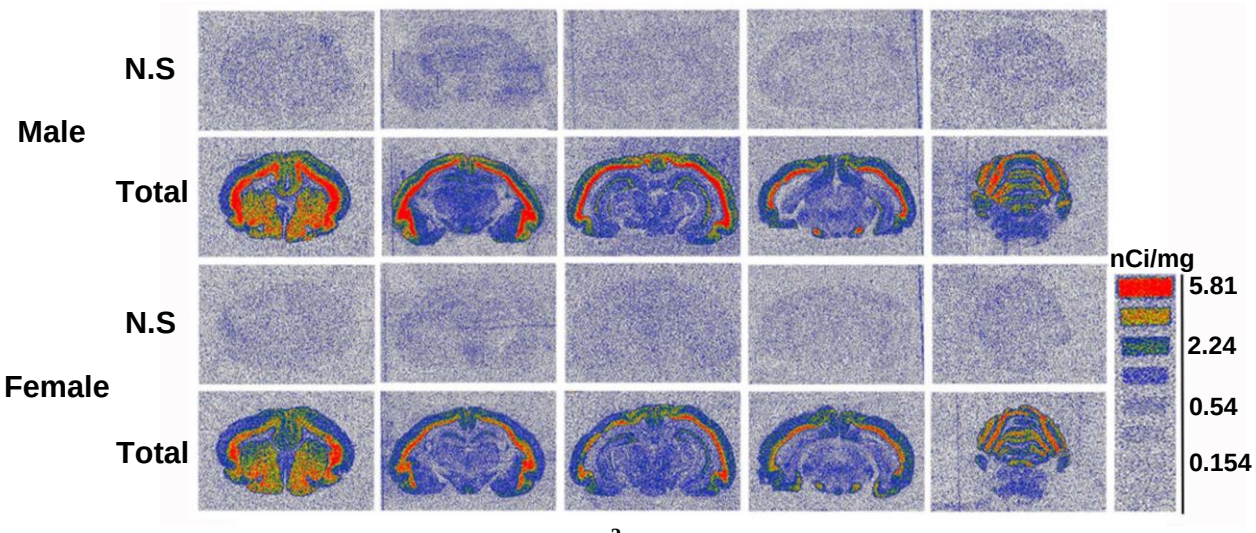

Figure 1. Psedudo-color autoradiograms of [^{3}H]U69593 (~5 nM) binding to the kappa opioid receptor in coronal brain sections of male and female guinea pig brain. Sections were incubated with ~5 nM [3H]U69,593 for 1 hour at 25°C, washed, dried and exposed to tritium-sensitive phosphor screens. Non-specific binding (N.S) measured in the presence of 10 μM naloxone. Exposure time was 3 weeks. The color bars are pseudo-color representation of black and white screen images in fmol/mg tissue equivalents. Male and female sections were processed in parallel throughout binding experiments and for development of autoradiograms.

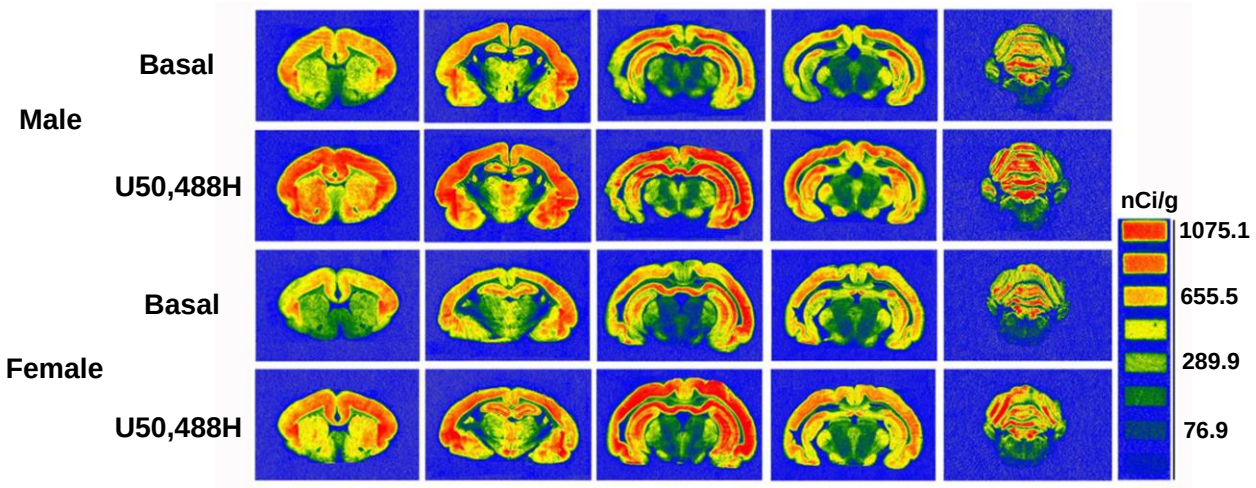

Figure 2. Pseudo-color autoradiograms of U50,488H-stimulated [^{35}S]GTPγS binding in coronal sections of male and female guinea pig brain. Sections were incubated with ~0.04 nM [^{35}S]GTPγS and 2 mM GDP, in the absence (Basal) or presence of 10 μM of U50,488H. Exposure time was 48 hours. The color bars are pseudo-color representations of black and white screen images in nCi/g tissue equivalents. Male and female sections were processed in parallel throughout [^{35}S]GTPγS binding experiments and for development of autoradiograms.

Table 1. Quantitative autoradiography of [^{3}H]U69,593 binding to the KOPR in male and female guinea pig brains.

Region	[^{3}H]U69,593 receptor binding fmol/mg)	
	Males	Females
Cingulate Cortex	94.8 ± 5.5	85.8 ± 1.9
Motor Cortex		
Superficial layers	76.8 ± 3.8	77.3 ± 4.5
Deep layers	128.5 ± 10.8	127.7 ± 6.6
Somatosensory Cortex		
Superficial layers	70.6 ± 3.1	65.4 ± 3.3
Deep layers	176.5 ± 10.6	148.2 ± 3.2*
Insular Cortex		
Superficial layers	97.2 ± 3.8	87.8 ± 1.7
Deep layers	270.9 ± 12.8	238.1 ± 6.0*
Caudate putamen	111.0 ± 3.8	110.2 ± 6.1
Claustrum	262.6 ± 10.0	235.8 ± 6.5*
Endopiriform nucleus	270.9 ± 13.1	238.9 ± 4.6*
Nucleus accumbens		
Core	138.3 ± 3.8	139.3 ± 5.1
Shell	189.2 ± 6.2	184.2 ± 7.9
septal	47.5 ± 2.7	44.9 ± 5.6
Hippocampus	37.4 ± 1.4	34.5 ± 2.4
Amygdala	39.2 ± 4.0	38.2 ± 1.3
Thalamus	20.9 ± 1.2	21.4 ± 0.9
Hypothalamus	39.8 ± 2.8	39.7 ± 3.5
Dentate gyrus	45.4 ± 1.7	40.8 ± 2.3
Periaqueductal gray	41.6 ± 2.1	35.9 ± 1.2*
Substantial nigra	122.4 ± 6.9	99.8 ± 6.2*
Medial geniculate nucleus	41.1 ± 1.6	40.4 ± 1.7
Cerebellum	71.1 ± 7.5	71.2 ± 3.5

Quantitation were made from both left and right sides of each section. Data are the mean ± S.E.M. of six animals, four or five sections from each brain. *$p<0.05$ different from male guinea pigs by the nonpaired two-tailed Student's *t* test.

Table 2. Quantitative autoradiography of U50,488H-stimulated [^{35}S]GTPγS binding in male and female guinea pig brains.

Region	Basal		Net U50,488H-stimulated GTPγS binding (nCi/g)	
	Male	Female	Male	Female
Cingulate Cortex	1587.1 ± 59.4	1484.0 ± 334.4	411.1 ± 64.1	334.4 ± 36.6
Motor Cortex				
Superficial layers	1854.3 ± 55.9	1730.6 ± 87.2	365.1 ± 61.6	258.7 ± 24.6
Deep layers	2090.3 ± 57.8	2006.7 ± 139.3	577.1 ± 86.2	441.8 ± 55.2
Somatosensory Cortex				
Superficial layers	1873.5 ± 51.1	1724.6 ± 92.0	304.5 ± 42.1	168.4 ± 37.5*
Deep layers	2078.6 ± 50.5	1980.0 ± 118.7	720.6 ± 75.9	434.1 ± 52.2*
Insular Cortex				
Superficial layers	1943.6 ± 79.3	1819.9 ± 90.5	417.1 ± 56.4	237.2 ± 52.9*
Deep layers	2395.4 ± 74.2	2262.7 ± 110.4	846.3 ± 97.4	465.6 ± 44.8**
Caudate putamen	927.3 ± 9.9	862.2 ± 54.1	309.6 ± 29.9	219.2 ± 20.6*
Claustrum	2188.8 ± 136.9	1904.4 ± 120.2	548.4 ± 47.7	306.9 ± 43.7**
Endopiriform nucleus	1485.6 ± 44.4	1372.2 ± 36.9	485.7 ± 80.7	336.7 ± 46.5
Nucleus accumbens				
Core	888.2 ± 19.3	824.9 ± 89.3	352.1 ± 33.5	294.1 ± 56.4
Shell	1027.9 ± 42.7	861.6 ± 84.2	323.1 ± 45.8	390.7 ± 46.8
septal	964.4 ± 46.9	897.1 ± 84.2	220.6 ± 72.8	175.9 ± 33.9
Hippocampus	1222.6 ± 61.8	1213.1 ± 104.9	68.1 ± 22.9	48.7 ± 19.1
Amygdala	928.0 ± 29.9	813.0 ± 77.5	126.4 ± 33.8	64.5 ± 26.2
Thalamus	688.9 ± 54.3	675.1 ± 68.2	44.6 ± 12.8	52.1 ± 13.9
Hypothalamus	661.8 ± 54.7	560.5 ± 51.6	178.3 ± 35.1	258.9 ± 26.6
Dentate gyrus	1042.3 ± 48.2	1016.8 ± 83.0	140.6 ± 20.3	250.9 ± 35.9*
Periaqueductal gray	541.7 ± 25.1	538.2 ± 12.8	171.2 ± 22.3	125.8 ± 4.0
Substantial nigra	683.5 ± 30.7	654.3 ± 21.6	343.5 ± 47.3	332.5 ± 24.9
Medial geneculate nucleus	562.9 ± 47.8	630.8 ± 42.4	131.3 ± 25.0	60.8 ± 12.0*
Cerebellum	426.8 ± 28.5	438.0 ± 10.7	53.9 ± 7.9	25.2 ± 8.4*

Quantitation was made from both left and right sides of each section. Data are mean ± S.E.M. of six animals, five sections from each brain. *$p<0.05$, **$p<0.01$ different from male guinea pigs by the nonpaired two-tailed Student's *t* test

Table 3. Quantitation of Phospho-p44/42 MAPK immunopositive labeling. Bright-field images of coronal sections were analyzed. Quantitation was performed using NIH ImageJ 1.42 software. Prior to quantification, a threshold level was chosen from a 256-level gray scale. The threshold was selected to allow detection of nuclei, cells and fibers stained with moderate to high intensity, while suppressing lightly stained nuclei, cells and fibers. An ROI was defined for each select brain region and used to sample from each region. Bilateral counts of phospho-p44/42 MAPK immunopositive cells and averages from 1-4 sections, depending on the brain region, were calculated for each animal with each animal representing a single observation. Comparisons between sex and treatment groups were made. Data represents mean (±sem). Male-saline (n = 3), Male-U50,488 (n = 3-4), Male-U50,488+nBNI (n = 3-4), Male-saline+nBNI (n = 3) Female-saline (n = 4-5), and Female-U50,488 (n = 5), where each n is an independent animal. *, $p < 0.05$, **, $p < 0.01$ vs saline, main effect of treatment (by 2 x 2 ANOVA); $^{+}$, $p < 0.05$, $^{++}$ $p < 0.01$ vs male, main effect of sex (by 2 x 2 ANOVA); $^{\wedge}$, $p < 0.05$ vs same-sex, significant interaction (by 2 x 2 ANOVA); §, $p < 0.05$ vs male-U50,488H same region (Student's t-test) ; $^{\$}$ $p < 0.05$, vs male-saline same region (Student's t-test).

Brain region	Female		Male			
	Saline	U50,488	Saline	U50,488	nBNI + saline	nBNI + U50,488
Cingulate cortex	9.4 ± 4.6	1.8 ± 1.4	7.0 ± 2.9	3.0 ± 1.2	3.4 ± 1.3	1.1 ± 0.9
Infralimbic cortex	12.0 ± 3.0	5.4 ± 1.9*	14 ± 6.6	5.0 ± 1.5*	3.8 ± 1.1	2.5 ± 1.8
Dorsal pendicular cortex	22.4 ± 6.2	13.0 ± 3.4	18.0 ± 5.1	12.7 ± 4.3	10.7 ± 0.9	1.3 ± 0.8§
Ventrolateral orbital cortex	7.0 ± 2.1	1.7 ± 0.9**	7.7 ± 2.2	0.7 ± 0.3**	4.8 ± 1.1	0.1 ± 0.1
Lateral orbital cortex	8.6 ± 1.8	4.2 ± 0.9**	6.7 ± 1.8	1.0 ± 0.6**	5.0 ± 1.6	0.5 ± 0.5
Claustrum	12.6 ± 7.3	15.8 ± 3.9	5.3 ± 1.5	21.7 ± 4.9	5.9 ± 1.1	2.0 ± 0.9§
Nucleus accumbens	11.6 ± 4.2	16.2 ± 8.1	1.0 ± 0.0	25.3 ± 3.7	2.9 ± 1.2	1.3 ± 0.8§
Lateral septum (dorsal)	26.8 ± 5.8	23.0 ± 2.1	26.3 ± 5.8	25.3 ± 8.7	18.1 ± 2.7	6.8 ± 2.7
Lateral septum (ventral)	25.6 ± 4.6	26.2 ± 3.9	28.3 ± 10.8	18.3 ± 5.2	22.3 ± 3.3	8.5 ± 1.9
Paraventricular nucleus	80.6 ± 19.5	62.8 ± 9.8	32.6 ± 9.7	81.9 ± 9.5$^{\wedge}$	29.9 ± 6.4	44.3 ± 4.1§
Ventromedial hypothalamus	35.4 ± 6.5	34.2 ± 2.0	29.9 ± 5.6	36.8 ± 5.0	15.2 ± 5.2	24.4 ± 7.3
Central amygdala	38.2 ± 8.6	63.4 ± 16.5*	36.7 ± 3.0	93.5 ± 15.9*	25.8 ± 3.8	39.5 ± 4.8§
Medial amygdala	31.4 ± 6.6	45.2 ± 7.6**	25.0 ± 6.1	54.0 ± 2.6**	20 .0 ± 3.8	22.4 ± 5.0§
Basolateral amygdala	31.0 ± 5.2^{+}	16.4 ± 3.7$^{*,+}$	11.0 ± 0.6	17.3 ± 1.4*	22.5 ± 2.8$^{\$}$	10.0 ± 2.7
Thalamus	31.6 ± 3.4	79.4 ± 14.7**	25.3 ± 7.3	86.0 ± 8.2**	24.4 ± 8.7	35.8 ± 5.9§
Periaqueductal gray region	38.3 ± 21.4	108.0 ± 24.7*	17.7 ± 7.2	60.3 ± 26.8*	12.4 ± 4.9	30.6 ± 16.5
Lateral PAG	5.6 ± 2.8	24.2 ± 4.5*	2.3 ± 0.3	17.8 ± 6.5*	1.9 ± 0.5	6.2 ± 2.8
Dorsal raphe nuclei	50.8 ± 11.0^{++}	90.6 ± 12.3$^{**,++}$	7.0 ± 2.3	50.8 ± 10.7**	7.9 ± 4.1	21.3 ± 8.3

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
__x___ 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, and an abbreviated title of the

publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

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. Sex difference in κ-opioid receptor (KOPR)-mediated behaviors, brain region KOPR level and KOPR-mediated guanosine 5'-O-(3-[^{35}S]thiotriphosphate) binding in the guinea pig.	Wang YJ, Rasakham K, Huang P, Chudnovskaya D, Cowan A, and Liu-Chen LY	*J.Pharmacol. Exp.Ther.*	May 2011	☐Submitted ☐Accepted X 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.

We have found that male guinea pigs show higher degrees of kappa opioid receptor-mediated abnormal postures/sedation and greater antinociceptive responses in the paw pressure test than females. In contrast, females show greater kappa opioid receptor-mediated inhibition of hyperactivity caused by cocaine. Therefore, there are sex differences in pharmacological responses to kappa opioid agonists in the guinea pig. This study has implications for clinical use of kappa opioid receptor agonists.

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___x
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. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

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Professor, Pharmacology
Department of Pharmacology
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Educational Background:
BS, National Taiwan University, Taipei, Taiwan 1976
MS, University of Minnesota (Mayo Clinic), Rochester, MN 1980
PhD, Massachusetts Institue of Technology, Cambridge, MA 1983
Postdoctoral Fellowship, Massachusetts General Hospital/Harvard Medical School, Boston, MA 1984

Research Interests:
Biochemistry and molecular and cellular pharmacology of opioid receptors.
Mechanisms underlying drug abuse.

PUBMED PUBLICATIONS:
Recent Medically Related Publications, Obtained from PubMed (Click on PubMed ID to view abstract)

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