Regulated Research Institutional/Industrial Setting Form (1C)

This form must be completed AFTER experimentation by the adult supervising the student research conducted in a regulated research institution, industrial setting or any work site other than home, school or field.

Student's Name(s) Chelsea Pan

Title of Project Dysregulation of dopamine-2 receptor with neuronal deficits underlies loss of control in cocaine addiction

To be completed by the Supervising Adult in the Setting (NOT the Student(s)) after experimentation:
(Responses must be on the form as it is required to be displayed at student's project booth; please do not print double-sided.)

The student(s) conducted research at my work site:

1. Did you or your proxy (e.g. graduate student, postdoc, employee) mentor or provide substantial guidance to the student researcher? ☑ Yes ☐ No
   a. If no, describe your and/or your institution's role with the student researcher and his/her project (e.g. supervised use of equipment on site without ongoing mentorship and sign below.

   b. If yes, complete questions 2 –5.

2. Is the student's research project a subset of your ongoing research or work? ☑ Yes ☐ No
   Use questions 3, 4 and 5 to detail how the student's project was similar and/or different from ongoing research or work at your site.

3. Describe the independence and creativity with which the student:
   a. developed the hypotheses or engineering goals for the research project

      The parental NIH project is to study how cocaine addiction affects the brain from genomic expression to brain function. In vivo imaging was performed at Stony Brook Univ (Kevin Clare). Ex vivo study here was not originally proposed. When Chelsea was introduced to me by Kevin (co-mentor), she was very interested in by questions such as "What receptors are involved if someone is addicted to cocaine?", "how do their expressions change?" As she had been trained in ex vivo imaging, we thought it would be a perfect project that she volunteered to image cocaine-induced changes in dopamine receptor 2 in neurons ex vivo using the existing brain samples of our project to confirm our in vivo imaging findings. So, this project was initialized by addressing her questions and she performed the ex vivo study independently.

   b. designed the methodology for his/her research project

      Unlike other high-school students I have supervised, Chelsea was already well trained for the ex vivo methodologies needed for her project. I suggested her to search for some relevant scientific papers to read and propose possible approaches to address the questions. Based on the methods she proposed in the lab meeting, I agreed that she should follow her idea to combine intrinsic Dr2-GFP fluorescence and immunohistochemistry staining (IHC) for signal enhancement by taking advantage of brain samples of our studies that were from a Drd2-GFP expressing transgenic mouse line.
      She performed the ex vivo studies independently. Indeed, the method worked well for her project.

   c. analyzed and interpreted data

      Chelsea carried out all data analysis independently.
      Initially, she counted the cell numbers by hand, which was time consuming. Later on, she worked with Kevin to modify an image program in ImageJ to enable automatic cell counting. When presenting the project progress to the lab, she interpreted her results with good understanding and organization. Then, we worked together to refine her results and interpretations.

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Regulated Research Institutional/Industrial Setting Form (1C) Continued

Student's Name(s) Chelsea Pan

4. Detail the student's role in conducting the research (e.g. data collection, specific procedures performed). Differentiate what the student observed and what the student actually did.

Chelsea was provided with the whole mouse brains fixed in paraformaldehyde after Kevin's in vivo studies. She performed all the ex vivo imaging procedures: cryoprotection of these brains with sucrose, embedded the brain tissues, and frozed them for cryostat sectioning into 50um sections and mounted them onto microscope slides. She performed immunohistochemistry staining to enhance the GFP fluorescence (green) and to all neurons with NeuN - a marker for neuronal nuclei (red). She then imaged the sections under a fluorescence microscope. After imaging, she quantified the number of GFP+ cells (D2r neurons) and NeuN+ cells (all neurons) in each image using a custom program in ImageJ and performed statistical tests and presented her findings to the lab. She actually did all the ex vivo work independently.

5. Did the student(s) work on the project as part of a group? ☐ Yes ☑ No
If yes, how many individuals were in the group and who were they (e.g. high school students, graduate students, faculty, professional researchers)?

Under Kevin and my supervision, she worked independently on her project (ex vivo imaging studies), which included sectioning mouse brain tissue, performing immunohistochemistry, capturing fluorescence microscope images, and analyzing data and presenting results in our group meetings for discussions.

I attest that the student has conducted the work as indicated above and that any required review and approval by institutional regulatory board (IRB/IACUC/IBC) has been obtained. Copies are attached if applicable.
I further acknowledge that the student will be presenting this work publicly in competition and I have communicated with the student research regarding any requirements for my review and/or restrictions of what is publicized.

Carl (Zhicheng) Lin	Assistant Professor
Supervising Adult's Printed Name / Signature	Title
Department of Psychiatry, Harvard Medical School	1/9/2020
Institution	Date Signed (must be after experimentation) (mm/dd/yy)
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