Guidelines for Correlated Multi-Scale Imaging Center project application

Overall Aim

The Center for Cannabis, Cannabinoids and Addiction supports the substance use disorder research community to study molecular changes in association with these disorders. We will provide know-how for molecular imaging in a *brain circuit*-, *cell-type*, and *subcellular compartment*- and *nanodomain*-specific manner that accompanies the different aspects of substance use disorders such as intoxication, craving, or withdrawal.

Eligibility

1. Must be trainees and/or PIs currently working in or moving into the substance use disorder field.

2. Have a well-developed research plan designed to address significant questions related to substance use disorders and/or strategy to provide preliminary data for grant applications.

3. Provide a clear rationale on how the experimental data generated by the imaging core will address a research question relating to addiction.

4. Identify a qualified scientist from the PI's lab who can physically be in Bloomington for the duration of the experiment.

5. Identify a clear strategy to analyze and summarize data in a timely manner.

6. Agree to share the data generated with the greater scientific community once results are published.

Investigators are strongly encouraged to consult with Drs. Barna and Katona before submitting a proposal to ensure compatibility of the proposal with the Core's capabilities.

Proposal Review

Each proposal will first be reviewed by the core PI for suitability. Proposals deemed suitable will be reviewed by the C3A steering committee. Drs. Barna and Katona will then collaborate with the steering committee to select projects based on scientific value, predicted impact on the substance use disorder field, and feasibility. All applicants will receive feedback on their proposals and will

be offered the opportunity to be mentored by a C3A PI or Affiliate in revised applications to the C3A. About three multi-scale imaging projects can be accommodated per year (July 1 – June 30).

Proposals will be ranked by the following criteria:

1. A clear rationale on how the proposed research project will benefit from access to the MSIC core and will address substance use disorders.

2. A well thought-out experimental plan on how data generated from the MSIC core will benefit a grant proposal and/or provide key data for a manuscript in preparation.

3. The impact of data generated by the MSIC core on the applicant's ongoing research program. Lower priority will be given to proposals that merely supplement ongoing research.

4. Data from MSIC core will be critical for the PI's NIDA (or other relevant funding agency) grant application.

5. Readiness to conduct the proposed experiment, such as the availability of essential mouse lines and/or key reagents for the proposed experiment.

6. A clear plan and commitment to execute the proposed experiment and availability of an experienced researcher from the PI's lab to perform the experiments, data acquisition, analysis and visualization with the supervision of C3A experts.

Specific criteria for Correlated Multi-Scale Imaging (MSIC-STORM) - proposals:

Please submit a 3-page-long application outlining the rationale and the proposed experimental details of your project. The application should contain a graphical abstract, some references, and a paragraph about how the project would help existing or future NIH grant applications of the PI. A prerequisite for the application is that the investigator will need to demonstrate the availability of a specific and sensitive labeling probe (antibody or small molecule) and access to appropriate tools for control experiments (KO mice, receptor antagonists, enzyme inhibitors etc). The application should also contain a power analysis to estimate the necessary sample size for the project. This analysis needs to take into account that investigating molecular changes in both sexes is advised.

In the application, the investigator also needs to briefly explain the substance use disorder model that is applied in their lab for the specific project. The experimental animals will need to be perfused in the applicant's lab at the time point(s) relevant for the experimental goal. We are glad to give advice, share our lab protocols and provide a brief one-day presentation training at IU of our perfusion protocol, if it would be helpful.

Next, the application should provide a detailed structure of the experimental plan based on the following workflow:

1. *Which brain circuit will be analyzed by the Slide Scanner?*

   As Step 1, it is advised to determine which addiction-related brain regions exhibit the strongest changes in the levels of the target protein by using a systematic regional analysis by the MIDI-III Slide Scanner.

2. *Which cell-type will be investigated with the A1 Confocal Microscope?*

   As Step 2, it is advised to determine which cell types contain the target protein. Visualization of this cell-type is possible by neurochemical markers and immunolabeling, or cell-type-specific expression of a fluorescent protein.

3. *Which subcellular compartment will be studied with the A1 Confocal Microscope?*

   As Step 3, it is important to determine which subcellular compartments may be the most relevant for the molecular changes associated with the substance use disorder model (axon terminal, axon, axon initial segment, cell body, dendritic shaft, dendritic spine; or different compartments of astrocytes, oligodendrocytes, microglia). Changes in immunolabeling intensity can be due to cellular (sprouting or pruning) alterations or specific molecular adaptations without a change in morphological parameters. This step will be carried out by a confocal microscopy analysis.

4. *Which target protein will be studied in a nanodomain-specific manner by correlative confocal and STORM microscopy on the combined N-STORM and C2 confocal setup?*

   As Step 4, it is important to determine the nanoscale molecular changes associated with the substance use disorder paradigm. The target molecule will be visualized by immunolabeling or by pharmacolabeling, whereas the target profile (cell-type and subcellular compartment) will be visualized by immunolabeling. Correlated confocal and STORM imaging will be used to determine to establish the nanoscale molecular adaptations in the respective target profile.

Before preparing the application, please consult Laszlo Barna (lbarna@iu.edu) and Istvan Katona (ikatona@iu.edu), who will help you with further questions and help to optimize your experimental workflow.

Submissions are open from 01/01/2024.

Please send your application.

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Made possible by NIDA, P30 DA056140