Center for Study of Opioid Receptors and Drugs of Abuse (CSORDA)

The research objectives of CSORDA are to gain insights into the mechanisms of action of endogenous opioids and opioid drugs at their cognate receptors with the goal of discerning fundamental processes contributing to behaviors such as analgesia, addiction, tolerance and withdrawal. The current focus **(Abstract)** is on the circuitry regulating dysphoric states and relapse following abstinence of opiate drugs including in different susceptibility models including neuropathic pain and PTSD (CSORDA PUBLICATIONS).

CURRENT RESEARCH PROJECTS:

Project I (Abstract): Genetic Dissection of Striatal Indirect-Pathway in Opioid Withdrawal Aversion (Nigel Maidment and William Yang).

Project II (Abstract): Mu Opioid receptors in Habenular Networks: Reward and/or Aversion? (Brigitte Kieffer).

Project III (Abstract): Impact of Chronic Pain on Circuitry Involved in Opioid Self-Administration Behaviors (Chris Evans, Wendy Walwyn and Catherine Cahill).

Project IV (Abstract): Bidirectional Comorbidity Between Fear Sensitization and Opioid Reward (Michael Fanselow).

CSORDA CORES:

Administrative Core (Chris Evans, Nigel Maidment and Eydie London).

CSORDA Advisory board, (Bernard Balleine, Charles Chavkin, Anto Bonci, Pat Levitt, Erik Nestler and Peter Whybrow ex-officio)

Technical Advancement Core: Animal resting-state MRI (Brigitte Kieffer) multilectrode array recording (Sotiris Mansmanidis), transcript and gene profiling (Giovanni Coppola and William Yang) optogenetics (Wendy Walwyn) cellular calcium imaging (Peyman Golshani).

Mouse Animal Breeding Core (Wendy Walwyn and William Yang),

Pilot Program Core (Eydie London)

The overall mission of CSORDA is to enhance the understanding of the mammalian opioid system thereby providing strategies for improving the clinical use of opioids and treatments for addiction.

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ABSTRACT (Overall)

Addiction to therapeutic opioid drugs and heroin has seen a marked increase in the US during the past two decades. In 2014, therapeutic opioid overdose and heroin were responsible for 18,893 and 10,574 deaths respectively. Opioid overdose is the primary driver for drug poisoning, being the leading cause of accidental death in the US, with 47,055 fatalities in 2014. The UCLA Center for Study of Opioid Receptors and Drugs of Abuse (CSORDA) has a focused multidisciplinary preclinical opioid research program with a broader educational and outreach mission in the area of addiction. Mu opioid receptors are targets for many addictive disorders since they are key components for mediating the rewarding effects of opiates, nicotine, cannabinoids, alcohol and food. CSORDA's research program focuses on elucidating the circuitry and cell-specific adaptations underlying addiction-related behaviors mediated by mu opioid receptors. This CSORDA renewal application (years 31-36) focuses on understanding the circuitry regulating dysphoric states and investigates different opiate addiction susceptibility models, including neuropathic pain, opioid withdrawal and PTSD. The research plan will build upon progress during the past funding period by incorporating several CSORDA-developed innovative genetic mouse models, findings with regards to resting state fMRI imaging, as well as the elucidation of circuitry regulating opioid reward via neuroinflammation and perturbation of D2 enkephalinergic systems. The renewal will use a model of PTSD to examine the marked comorbidity of this disorder with addiction to drugs reliant on the endogenous opioid system for reward-related behaviors. To maintain CSORDA as a technically cutting edge and innovative Center, we have created a Technical Advancement Core (TA-Core) that will enable CSORDA's research plan to incorporate new technologies optimized and vetted for CSORDA research. Four Research Projects are proposed that are highly interactive, both thematically and technically, and which use shared models, reagents and methods. Projects will focus on different brain circuitry associated with addiction, including the mesolimbic VTA striatal reward system (Projects I and III), the habenula (Projects II and III) and the amygdala (Project IV). Research Projects will explore the modulation of circuitry in models of chronic pain (Project III), withdrawal and depression (Projects I, II and III) and PTSD (Project IV). The research will employ mouse genetics and behavioral analysis combined with electrophysiology, optogenetics, transcript analysis and MRI imaging. In addition to the TA-Core, CSORDA will support an Animal Breeding Core (AB-Core) supplying all CSORDA Projects with mouse models and sharing reagents with the research community. The Administrative Core and CSORDA Advisory Board, consisting of Drs. Bernard Balleine, Antonello Bonci, Charles Chavkin, Pat Levitt, Eric Nestler and Peter Whybrow ex-officio, will provide programmatic oversight and coordinate training, outreach and a vigorous Pilot Program.

ABSTRACT (Project I)

A major force driving relapse in drug addiction, including opiate addiction, is the almost intolerable aversive effects of drug withdrawal. The biological bases of learned aversion to the drug withdrawal state have important mechanistic overlap with those for aversion to non-drug-related noxious stimuli (e.g. bitter taste, footshock, social defeat, traumatic stress) and involve multiple brain regions. Among them, the interconnected basal ganglia (BG) nuclei, including nucleus accumbens (NAc), ventral pallidum (VP) and dopaminergic (DA) neurons in the ventral tegmental areas (VTA) appear to be particularly crucial. In this proposal, we will focus our study on the NAc, not only because prior studies have implicated this brain region as a critical substrate for both rewarding and aversive effects of opiates, but also because recent studies suggest a particular neuronal cell type in the NAc, the D2 medium spiny neuron (D2-MSN), is essential to establishment of avoidance behaviors elicited by noxious environmental stimuli as well as the intense aversive effects of opiate withdrawal. Since manipulating the activities of D2-MSNs using the latest chemogenetic or optogenetic tools have demonstrated pivotal roles of these neurons in aversive behaviors, we reason the critical next step is to dissect the roles of D2-MSNs in non-drug-related and opiate withdrawal-related aversion through genetic analyses of endogenous genes that are highly selectively expressed in D2-MSNs. To this end, we hypothesize three such genes - two receptors (Gpr6 and Adora2a) and one peptide precursor (pre-pro-enkephalin (Penk)) - play important roles in opiate withdrawal aversion. We propose that signaling through these two Gs-linked receptors, by opposing the signaling imparted by the Gi/o-coupled dopamine D2 receptor, is crucial for D2MSN mediation of both non-drug and opiate withdrawal-related aversion. To test this, we will investigate the behavioral and signaling effects of single or double deletion of these receptors. Conversely, our recent data demonstrate that pro-enkephalin-derived peptides in D2-MSNs play a crucial role in mediating an endogenous opioid hedonic tone, hence we will test the idea that removal of Penk in D2-MSNs will exacerbate the aversive effects of opiate withdrawal. Finally, we have obtained exciting preliminary data, using RNA-seq and network analyses, showing aberrant changes in NAc molecular networks upon the establishment of opiate dependence. We will apply such powerful integrative systems biology approaches to investigate whether genetic perturbation of key D2-MSN genes or opioid network genes alters the molecular signatures of opiate dependence.

ABSTRACT (Project II)

Addiction develops when recreational drug use switches to compulsive drug taking. While the former is predominantly motivated by reward seeking, the latter is also driven by other factors that include enhanced stress reactivity, aversive aspects of drug withdrawal and emergence of a negative affect upon protracted abstinence. In recent years the notion that reward and aversion processing engage overlapping brain circuits has been established, together with the concept of a reward/aversion network. The habenula (Hb) encodes both rewarding and aversive aspects of external stimuli, and may therefore represent a central integrator of reward/aversion circuits. Remarkably, the medial subdivision of habenula (MHb) shows highest density of mu opioid receptors (MORs) in the brain, but the role of this particular receptor population is unknown. This project will test the hypothesis that MORs expressed in the MHb regulate specific aspects of reward and aversion processes related to drug abuse.

We will capitalize on tools and preliminary findings from the previous funding period. In Aim 1, we will extensively characterize MOR-expressing neurons in the medial septum-MHb-interpeduncular nucleus (MS-MHb-IPN) pathway using viral tracers combined with knock-in MOR-mcherry, Cnrb4-Cre or novel MOR-Cre mice that we currently develop. This Aim will provide circuit-level understanding that will complement Aims 2 and 3. Aim 2 will identify behaviors, and underlying circuit mechanisms, controlled by MORs in the MHb. We will examine a range of reward/aversion behaviors potentially mediated at the Hb level (reward and reward-driven decision-making, morphine and nicotine withdrawal, aversion to morphine withdrawal and abstinence) using a novel conditional Cnrb4-MOR mouse line. Reduced physical morphine withdrawal has already been detected and DREADD approaches will be used to recapitulate this behavior, and possibly other phenotypes. Aim 3 will identify the causal impact of MOR and MOR-positive neuron activities in MS-MHB-IPN networks, and their broader impact on the brain. We will use pioneering functional magnetic resonance imaging (fMRI) in live mice, and further fMRI strategies developed in the Technical Advancement Core, to map brain-wide functional connectivity, seed-based connectional patterns and inter-node directionality in Cnrb4-MOR mice at rest and after morphine treatment. Consequences of DREADD-mediated stimulation of MOR+ neurons in the MHb will also be examined by fMRI in live animals. In sum, this proposal will reveal the role(s) of the densest and less-well studied MOR population. The three aims together will determine importance of these receptors in reward/aversion-related behaviors and elucidate the underlying circuit mechanisms. The project will also provide novel genetic mouse lines for CSORDA (Cnrb4-MOR, Project III) and the neuroscience community (MOR-Cre), and cutting edge noninvasive animal imaging that will be applicable within (PTSD model, Project IV) and outside of CSORDA.

ABSTRACT (Project III)

Opioid abuse in the USA has recently reached epidemic proportions, with 29,440 of the 47,055 fatalities from drug poisoning in 2014 attributable to either therapeutic opioids or heroin. Prescription opioids such as morphine, oxycodone and fentanyl as well as heroin are widely abused. The recent increase in opioid abuse is mirrored by a skyrocketing 500-1000% increase in the number of pain prescriptions over the last decade, although the abuse of therapeutic opioids is largely a result of diversion. In spite of the alarming statistics it must be acknowledged that pain requires and demands treatment. The role of pain in opioid addiction is murky; with some studies claiming addiction liability is enhanced by pain, yet others claiming that pain is protective. Technically challenging self-administration of morphine, oxycodone and remifentanil in mice (that were

developed during the past funding period) will be used to assess the influence of neuropathic pain on phases of the establishment of opioid self-administration followed by cycles of withdrawal (extinction) and relapse (reexposure).

Our preliminary data suggest that neuropathic pain does not alter initial oxycodone selfadministration but enhances drug-seeking during extinction and causes an enhanced escalation in drug taking during re-exposure. These findings will be expounded upon in Aims 1a and 1b using different opioid drugs and doses in self-administration protocols. The duration of time after sciatic nerve injury will be a variable since we have recently shown that pain symptoms can dissipate over time due to an increase in constitutive signaling of mu opioid receptors (MOR), yet negative affect continues to incubate over time. Given negative affect accompanies both chronic pain and opioid withdrawal, we will test in Aim1c if inhibiting neuroinflammation (published during the past funding period to be induced both by chronic opioid treatment and chronic pain), or kappa opioid receptors, (that we show in preliminary data have markedly increased function during chronic pain) will modify oxycodone reinforcement behaviors in animals with or without neuropathic pain. To identify MOR cell types and the striatal and/or habenula circuitry that are required for aspects of the opioid reward profile. Aim 2 will use conditional deletion or knock-in strategies to remove or insert MOR in striatal and extra-striatal neurons. Preliminary data shows marked differences in the self-administration profiles of these mice. For example, removing MOR from D1 neurons increases oxycodone consumption and drug seeking during extinction despite a complete absence of oxycodone-induced locomotor sensitization, while the converse is seen in mice lacking MORs on D2 neurons. Electrophysiological analyses will determine if pain alters properties of different striatal neurons following opioid self-administration. Finally, as D2 cells contribute to negative affect during withdrawal, we will use optogenetic stimulation of D2 neurons to show how these neurons modulate drug-seeking in the presence of pain. Together, Project III will assess the circuitry involved in different phases of opioid selfadministration

and its influence by different pain and dysphoric states.

ABSTRACT (Project IV)

People suffering from anxiety disorders such as post-traumatic stress disorder (PTSD) very often use and abuse reinforcing drugs such as opioids. Although the comorbidity of PTSD and substance abuse is exceedingly high, little is known about the neurobiological mechanisms that result in this comorbidity. By leveraging the Fanselow laboratory's development of a preclinical model of PTSD with the expertise within the Center for Study of Opioid Receptors and Drugs of Abuse (CSORDA) we plan to attack this question. Based on findings with our model we hypothesize that, during a single traumatic event, stress hormones (corticosterone/cortisol) and neuromodulators (acetylcholine) act in concert on amygdala neurons resulting in changes in neural plasticity within this brain structure. These changes result in the altered fear responses that characterize several aspects of PTSD. The amygdala also contains neurons that participate in the rewarding property of drugs and we hypothesize that trauma causes similar changes in those neurons and this leads to increased drug reward learning. Therefore, in Aim 1 of this proposal we will determine if manipulations that mitigate the potentiation of fear learning caused by trauma also mitigate alterations in drug responsivity following trauma. Aim 2 seeks to identify a set of amygdala neurons that are activated by BOTH trauma and drug exposure and using optogenetics tests if activity in these neurons is necessary for comorbidity. We will also assess how stress and drug experience alter gene expression patterns in the amygdala. While most of the focus on drug use/anxiety disorder comorbidity has focused on stress as a causal factor in altered drug taking, we have obtained preliminary data that the converse is also true. A history of drug use and withdrawal increases the impact that a future stressor has on fear processes. Aim 3 investigates potential mechanisms for the ability of drug exposure to adversely impact fear behavior. We will determine if mu-opioid or kappa-opioid receptors in the amygdala are necessary for morphine's effects on future stress reactivity. We will also use resting state fMRI, with an amygdala seed, to determine the overlap in the modifications of whole brain connectivity caused by drug exposure and by stress.

ABSTRACT (Administrative Core)

The primary role of the CSORDA Administrative Core is to provide an infrastructure for maintaining an

innovative, rigorous and collaborative center of excellence for addiction-related research, training and outreach. The Administrative Core provides programmatic structure, logistical support, and mediation of the Center's interactions with training programs, administrative offices, and scientific and governmental institutions. The Core is responsible for budgetary and scientific oversight (including scientific directions and rigor) of all CSORDA research, including the Pilot Program. The PI of CSORDA, Dr. Christopher Evans, directs the Administrative Core, and in his absence, the Associate Director, Dr. Nigel Maidment, serves in this capacity. The Core has a sophisticated and experienced Advisory Board to provide guidance on both administrative and scientific issues. The Core is staffed by Ms. Polly Segal, an experienced administrator who has been associated with CSORDA for the past 9 years.

Responsibilities of the Core include:

• Oversight and evaluation of the Center; including organizing leadership, meetings of the Advisory Board, Steering Committee and biweekly center meetings, implementing of leadership decisions, coordinating educational and outreach roles for the Center, monitoring progress and facilitating collaborative interactions.

• Oversight of the Pilot Program; including advertising for potential grantees, evaluating and selecting of proposals, and integrating with ongoing activities within CSORDA.

• Assurance that the Center conducts rigorous research that considers sex differences, confounds in techniques, procedures and reagents and uses appropriately powered studies and statistical analysis.

• Oversight of personnel operations; including hiring, advancements and promotions, recruitment and conflict resolution, including arbitration in the unlikely event of disagreement between co-leadership of Projects or Cores.

- Support for publications, grant preparations, seminars and meetings, travel and purchasing.
- Budgetary preparation, prioritization and monitoring of fiscal resources in all Projects, Cores and Pilots.
- Coordination of the sharing of resources generated by the Center.

The Administrative Core is essential to the efficient operation of CSORDA.

ABSTRACT (Technical Advancement Core)

The Technical Advancement Core (TA-Core) has the overall goal of providing critical and appropriate expertise to use new technologies within CSORDA Projects and Pilots, as well as to advance and optimize these technologies for addiction-related research. The Core will also provide consultation (through Dr. Art Arnold) regarding analysis of potential sex differences observed in the animal experiments. The primary areas of technological contributions to the Center are in transcriptomics and bioinformatics (William Yang and Giovanni Coppola) and in MRI imaging (Brigitte Kieffer), which is reflected by the majority of resource allocation to these areas. The Core will additionally provide expertise and technical development for miniaturized microscopes (Peyman Golshani), wireless optoprobe (Wendy Walwyn) and silicon microprobe multi-recording devices (Sotiris Masmanidis). The Core Leaders, (Drs. Yang and Golshani) are both productive researchers, highly collaborative and experienced in leadership of shared resources and will have oversight of the administrative aspects and reporting of the core

The Transcriptomics and Bioinformatics component will provide expertise for Projects I and IV as well as Pilot I and aid in characterization of mice for the AB-Core. The TA-Core leverages existing UCLA and NIHsupported

infrastructure at UCLA specifically developed to support neuroscience investigators with genetics, genomics, sequencing, and bioinformatics experiments, therefore providing CSORDA faculty with a depth of technical expertise.

The MRI imaging component will contribute to aims in Projects II and IV. This is an emerging technology to determine high-resolution brain region connectivity with the potential to guide future CSORDA research to areas connected with the circuitry investigated in current CSORDA Projects.

The Miniaturized Microscope component will be utilized in Project I and Pilot IV for calcium imaging and striatum circuit analysis following opioid treatments. Development of the technology for addiction and CSORDA-related research will focus on combining the miniaturized microscopes with drug delivery systems for future experiments allowing direct analysis of neuronal networks by local drugs perfusion.

The Silicon Microprobe Multi-Recording Devices component will be utilized in Pilot III to assess modulation of striatal network response to natural rewards during opioid withdrawal. Development of the technology will employ optogenetics for cell-specific analysis and activity modulation of D1 and D2 Medium Spiny Neurons. The Wireless Optoprobe component will be utilized in Projects I, III and IV. The core will improve the battery, modify the design to include bilateral probes and alter the communication system to enhance range. The TA-Core will be integral to current and future technical approaches utilized by CSORDA.

ABSTRACT (Animal Breeding Core)

As all Center components will use genetically engineered mice, a central Animal Breeding Core (AB-Core) will be used to meet the animal needs of all Center Projects and Pilots and provide cost-effective, uniformly bred and monitored use of CSORDA animals. Additionally, the Core is a resource, particularly for mice which are mutant in opioid-genes, for providing animals for Pilots and research outside of CSORDA at UCLA, as well as many other institutions. The AB-Core was established in 2006 and developed a set of standardized procedures to accomplish this goal.

The functions of the AB-Core are as follows:

1. The AB-Core will coordinate with each Project, Pilot and the TA-Core to provide the required cohorts of male and female mice as needed. Planning, prioritization and sharing of animal breeding will be an agenda item at Steering Committee meetings.

2. The AB-Core will maintain each line under optimal breeding management practices.

3. Since multiple CSORDA research components utilize cross-bred lines, the AB-Core will ensure all lines are correctly maintained as congenic on the C57BL/6J background.

4. The AB-Core will follow the correct breeding strategies to ensure wild-type and mutant mice are bred as littermates.

5. The AB-Core will verify that correct patterns of expression are achieved following cre-mediated deletion in both males and females.

6. The AB-Core will coordinate mouse sharing, with a major role of the transfer of breeding pairs between CSORDA performance sites at UCLA and Dr. Kieffer at the Douglas Institute in Montreal.

ABSTRACT (Pilot Program)

The Pilot program has proven to be an important mechanism for the evolution of CSORDA by incorporating new and innovative project areas, new faculty participation, and new technical approaches. The program also has introduced talented UCLA faculty to addiction-related research. The Pilot Core will fund 4 Pilot Projects/year. Pilots have full access to CSORDA resources including the Animal Breeding Core (AB-Core) and the Technical Advancement Core (TA-Core). The TA-Core includes consultation with the Semel Biostatistics Core (SiStat) and Dr. Arthur Arnold for expertise and reagents for the study of sex differences. Logistical management, as well as budgetary and scientific oversight of the Pilot Core, will be the responsibility of the Administrative Core. The Pilot selection process involves a call for proposals and evaluation of the rationale, approach, and rigor of proposals by the Pilot Project Selection Committee, chaired and chosen by Dr. Edythe London. This committee selects project finalists, which are subsequently presented at a biweekly CSORDA meeting. The presentation provides the opportunity for initiating collaborations, as well as critiquing, and optimization of Pilot Projects. Funding is discussed at the Steering Committee leadership meetings, with the Directors making final decisions in consultation with Dr. London and the Advisory Board. Pilots will be funded according to their innovation, research excellence and rigor, as well as impact on substance abuse research. Given equivalent merit, priority will be given to projects most closely related to the theme of the Center or that offer new technologies or research for future CSORDA directions. Pilot progress will be monitored by presentations at a biweekly CSORDA meeting between 6 to 9 months after funding begins. Annual NIH Progress Reports provide documentation of progress. If a second year of funding is requested, a competitive application will be required. The Projects selected for funding during Year 1 of the renewal (with possibility of funding in Year 2) are: I) Pamela Kennedy, Assistant Professor in Psychology, will investigate a switch from goal-directed to habit learning following opioid drug withdrawal and a role for kappa opioid

receptors; II) Sotiris Masmanidis, Assistant Professor in Neurobiology, will use silicon microprobe recording technology developed by his group to investigate the perturbation of striatal physiology following withdrawal following chronic opioid administration; III) Kate Wassum, Assistant Professor in Psychology, will explore neural circuits underlying maladaptive reward-seeking decisions in opiate withdrawal; and IV) Carlos Cepeda, Research Professor in Psychiatry, will use miniaturized microscopes from the TA-Core to image calcium activity in the striata of freely-behaving mice following opioid administration. Sex differences will be considered in every Pilot. These 4 Projects all have potential to generate NIDA-related independent research programs or to be incorporated into future renewals of CSORDA. For Years 3-5, we will fund one or two translational (T1 or T2) Pilots, applying for cost sharing with the UCLA CTSI for more substantive funding. Project