IMPACT OF MATERNAL CIRCADIAN DISRUPTION ON SLEEP AND PFC FUNCTION IN OFFSPRING

Introduction: Modern society has brought changes in daily living that include 'around the clock' work schedules and a decoupling of sleep schedules from natural day/night cycles. In humans, shift work, jet lag, and sleep disruption lead to increased stress, metabolic deficits, poor mental health outcomes and decreased cognitive performance. Maternal biological rhythms directly impact offspring during the pre- and perinatal period[1,2]. Comorbidities associated with maternal sleep disruption are associated with negative fetal outcomes, but there is a paucity of literature assessing the direct impact of maternal sleep disruption on offspring[3]. *The <u>long-term</u> goal of my research is to identify the mechanisms by which developmental programming modulates daily rhythms and neurophysiology during adulthood.*

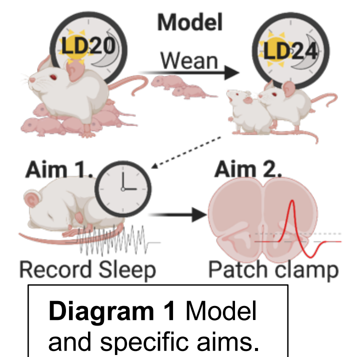

Diagram 1 Model and specific aims.

The prefrontal cortex (**PFC**) is critical for fear learning and extinction, cognitive tasks such as attention and working memory, and stress responses[4–6]. Maternal stress has profound effects on neurodevelopment in offspring and leads to changes in cognitive function, processing, and interconnectivity between the PFC and other brain regions[7]. Previous work from our lab demonstrates that environmental circadian desynchronization (**ECD**) induced by shortening the light/dark cycle to 10:10 (LD20) causes deficits in metabolism, PFC-mediated behaviors and decreases dendritic arborizations in layer II/III prelimbic (pl) PFC pyramidal neurons[8]. My preliminary data show that these same neurons undergo daily changes in excitability, with a hyperpolarized resting membrane potential (**RMP**) during the dark (subjective day) period, (**Fig.1A**) and that ECD disrupts pyramidal neuron function, including changes in RMP associated with the onset of the dark period (**Fig.1B**). My own previous work highlights that developmental programming has lifelong impacts on neural function[9]. However, *it is unknown* how ECD impacts sleep and PFC function in mice from ECD dams.

Hypothesis and Specific Aims

My *central hypothesis* is that maternal (m) ECD disrupts sleep in adult offspring and alters the function of layer II/III plPFC pyramidal neurons. I will test this with the following specific aims (**Diagram 1**):

Aim 1: Determine how maternal ECD impacts sleep patterns in adult offspring. We will approach this aim using *in vivo* sleep monitoring and manipulation of the maternal LD cycle.

Aim 2: Determine how maternal ECD impacts the function of PFC pyramidal neurons. We will approach this aim using *ex vivo* brain slice electrophysiology techniques with mice from Aim 1.

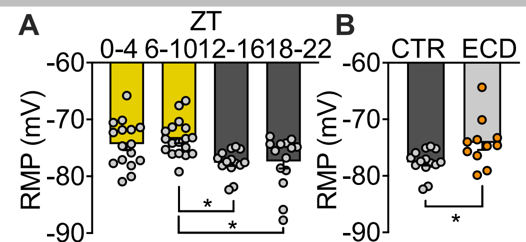

Fig.1 (***A***) RMP at four ZT bins from pyramidal neurons in CTR mice (12:12 LD). (***B***) RMP at ZT12-16 in CTR and ECD (10:10 LD, ZT calculated as 50min/hr) mice. *$*p<0.05$.*

Methods

<u>*Maternal ECD model*</u>: Individually housed 6-8wk old female C57BL/6J mice are placed in computer controlled light boxes set to control (CTR) LD24 or LD20 (ECD) for 3wks before introducing a male breeder previously housed at LD24. Male and female CTR/mECD pups are weaned into LD24 at postnatal day (P) 21.

<u>*Sleep recording*</u>: At P42-49 offspring are placed in a piezoelectric sleep monitoring system for 72h of continuous recording (Signal Solutions, Inc). This non-invasive approach has minimal outcome discrepancies from invasive EEG/EMG recordings[10], and the lab has used this approach to monitor sleep in mice (*data not shown*).

<u>*Brain Slice Electrophysiology*</u>*:* Following sleep recordings, mice are euthanized 1h prior to zeitgeber (ZT) bin 6-10 or ZT12-16, 2-5d after sleep recordings. 250µm coronal PFC slices are cut in a vibratome using a chilled and oxygenated NMDG cutting solution[11] then recovered in artificial cerebrospinal fluid (aCSF). Slices are placed under a microscope with gravity perfusion at 37°C. Recording pipettes (3-5MΩ) are backfilled with a K-gluconate internal solution. 0.2% lucifer yellow (LY) is added to confirm pyramidal shape and presence of an apical dendrite. Resting membrane potential (RMP) is measured in the current (I) clamp configuration sans I-injection. The $GABA_A$ antagonist bicuculline (30µM) is added to the aCSF for spontaneous excitatory postsynaptic current (sEPSC) recordings (V_{Hold}=-70mV). IV-curves are ran with 10mV steps (-120 to 30mV). Evoked action potentials (eAP) are measured by 10pA I-injection steps (-50 to 150pA).

Aim 1: Determine how maternal ECD impacts sleep patterns in adult offspring

The *rationale* for this aim is that perturbations in the maternal environment have lifelong consequences for offspring, including changes in daily rhythms[12]. However, how mECD impacts sleep in adult offspring is *unknown.*

Study 1.1: We will test the impact of mECD on sleep of adult offspring by maintaining (4) CTR and (4) ECD dams. Pups will be group housed with same-sex littermates at weaning. Mice will be individually housed in the piezo sleep monitoring system for a 48hr acclimation period, followed by 72h of sleep recording. After recording, mice will be placed back into their home cages in preparation for **Aim 2**.

Aim 2: Determine how maternal ECD impacts the function of PFC pyramidal neurons

The *rationale* for this aim is that ECD causes altered architecture/morphology of PFC pyramidal neurons[8] and my preliminary data show that ECD disrupts their resting membrane potential (**Fig.1B**) and presynaptic excitatory inputs (**Fig.2A,B**). However, the effect of mECD on the function of these neurons in adult offspring is *unknown*.

Patch-clamp recordings will be performed at ZT6-10 and ZT12-16 in male and female CTR/mECD mice.
Study 2.1: We will test the impact of mECD on pyramidal neuron function by measuring:
1) RMP, Rheobase, eAP frequency, AP threshold, amplitude, half-width, area, rise and decay time.
2) IV relationship: Inward/outward rectifying currents, I-density, and conductance at inactive/active states.
3) Cell properties including membrane resistance (R_{series}), capacitance, and R_{input} (recording quality control)
Study 2.2: We will test the impact of mECD on excitatory synaptic inputs by measuring sEPSC frequency, amplitude, and decay tau.

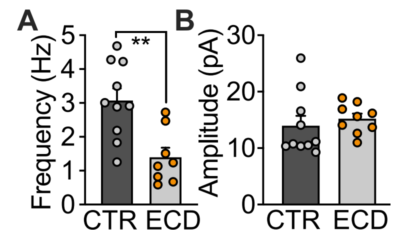

Fig.2 (***A***) sEPSC frequency and (***B***) amplitude pyramidal neurons at ZT12-16 in CTR and ECD mice. *Student t-test. **p<0.01.*

Statement of Need: Funds provided by this award will support the collection of pilot data to support career development grant applications, within PI Roberts' area of interest, at NIH (K01) and SRS (Career Development Award). Dr. Karatsoreos, the applicant's mentor, has grants (NIH, NSF, Keck) that address questions related to chronobiology, stress, and metabolism. However, the applicant has a specific interest in early life development and its impact on sleep, metabolism, and synaptic function. These funds are needed to bridge the gap between the applicant's direct interest and the projects funded by their mentor.

Impact on Career Development: To date, the applicant has obtained two travel grants, a two-year grant during graduate school, published multiple papers, has two manuscripts under review, and is preparing two for submission (completed with his new mentor after just 1 year amid the pandemic). However, the applicant has not had success obtaining project funding at the postdoctoral level, which is critical for continuing their academic career. Successful completion of this proposal will result in three core outcomes for the applicant: 1) Bolster subsequent grant proposals with a robust preliminary dataset 2) Demonstrate to future grant reviewers the applicant's ability to obtain funding, and 3) Widen the scope of future work and publications by pairing pilot data from this award with data collected under the mentor's funding.

Budget Plan: Funds are requested for *Animal Housing* and *Patch-clamp Supplies*. Animal Use: This proposal uses mice charged at an institutional *per diem* rate of $1.89 per breeder cage and $0.90 per standard cage. We require 4 CTR and 4 mECD breeder cages maintained for ~56 days totaling: (4 CTR) x (4 mECD) x (56 days) x $1.89 = $846.72. Litters are weaned at P21 and euthanized prior to P56. Litters are split into 4 groups including male/female CTR/mECD offspring. We require 5 cages/group at 2 time points totaling: (5 cages) x (4 groups) x (2 time points) x (35 days) x $0.90 = $1,260.00. Total funds for *Animal Use* are $846.72 + $1,260.00 = 2106.72. *Patch-clamp Supplies*: Funds are requested for reagents used to prepare patch-clamp solutions. Total reagent costs (Sigma-Aldrich) are: $1,904.04. We request funds for recording pipette glass (*$112.00*), osmometer testing tubes (*$544.00*), and two mixed air (oxygenating solutions) and nitrogen (air table) tanks (*$280.00*) equaling: $936.00. Combined requested funds: ($846.72 + $1,260.00; animal use) + ($1,904.04 + $936.00; patch-clamp reagents/supplies) = **$4,946.76**. All other equipment/supplies are readily available in the Karatsoreos Lab.

References: **(1)** Abe, M. et al. Circadian Rhythms in Isolated Brain Regions. *J. Neurosci*. 22, 350–356 (2002). **(2)** Pires, GN. et al. Effects of sleep modulation during pregnancy in the mother and offspring. *J. Sleep Res.* 30, e13135 (2021). **(3)** Pien, GW & Schwab, RJ. Sleep Disorders During Pregnancy. *Sleep* 27, 1405–1417 (2004). **(4)** Popoli, M. et al. The stressed synapse. *Nat. Rev. Neurosci*. 13, 22–37 (2012). **(5)** Sotres-Bayon, F. et al. Brain Mechanisms of Fear Extinction. *Biol. Psychiatry* 60, 329–336 (2006). **(6)** Miller, EK & Cohen, JD. An Integrative Theory of PFC Function. *Annu. Rev. Neurosci*. 24, 167–202 (2001). **(7)** Van den Bergh, BRH. et al. Prenatal developmental origins of behavior and mental health. *Neurosci. Biobehav. Rev.* 117, 26–64 (2020). **(8)** Karatsoreos, IN. et al. Disruption of circadian clocks has ramifications for metabolism, brain, and behavior. *Proc. Natl. Acad. Sci.* 108, 1657–1662 (2011). **(9)** Roberts, BL. et al. Early overnutrition alters synaptic signaling and induces leptin resistance in arcuate POMC neurons. *Physiol. Behav*. 206, 166–174 (2019). **(10)** Mang, GM. et al. Evaluation of a Piezoelectric System as an Alternative to EEG/EMG Recordings in Mouse Sleep Studies. *Sleep* 37, 1383–1392 (2014). **(11)** Ting, JT. et al. Preparation of Acute Brain Slices Using an Optimized NMDG Protective Recovery Method. *JoVE J. Vis. Exp.* e53825 (2018) doi:10.3791/53825. **(12)** Cleal, JK. et al. Maternal Obesity during Pregnancy Alters Daily Activity and Feeding Cycles, and Hypothalamic Clock Gene Expression in Adult Male Mouse Offspring. *Int. J. Mol. Sci.* 20, (2019).