Neurophysiology of daily rhythms in the prefrontal cortex of male and female mice

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INTRODUCTION

Modern society has brought changes in daily living that are associated with negative health consequences. This includes 'around the clock' work schedules and a decoupling of sleep schedules from natural day/night cycles, which impact both physical and mental health. For instance, too little sleep, such as insomnia, is a predictor for poor neurocognitive health, and associated with hyperarousal of cognitive-emotional systems, and decreased performance on neurocognitive tests[1–4]. Disruption of normal circadian rhythms, such as those caused by shift-work and exposure to artificial lighting at night, also have negative consequences for physiological and psychological health[5–8].

The PFC is a key region in executive function and contributes to learning and memory, impulse control, fear extinction, and stress responses[9–11]. While the PFC seems less developed in rodent species, a combination of tracing and lesion studies have identified the prelimbic (PL) medial (m) PFC of the rodent as a close homolog[12,13]. The mPFC contains both excitatory pyramidal neurons and inhibitory GABAergic interneurons. These interneurons tightly regulate the dynamic activity of pyramidal neurons, controlling spike generation, timing, and throughput of incoming afferent information to these neurons[14]. Interneurons also regulate oscillatory synchronization within the PFC, which shows clear diurnal and circadian dependent changes in gene expression, and electroencephalographic activity[15–18].

It is unknown how daily rhythms impact neuronal activity in the PFC at the cellular and synaptic level.

Here we use patch-clamp electrophysiology techniques to record from layer II/III pyramidal neurons in the PL mPFC of male and female mice at 4 separate bins of zeitgeber time (ZT): 0-4, 6-10, 12-16, and 18-22. We used current and voltage-clamp configurations to determine changes in membrane potential, excitatory and inhibitory inputs, action potential kinetics.

RESULTS:

Time of day alters excitability of layer II/III mPFC pyramidal neurons in male mice

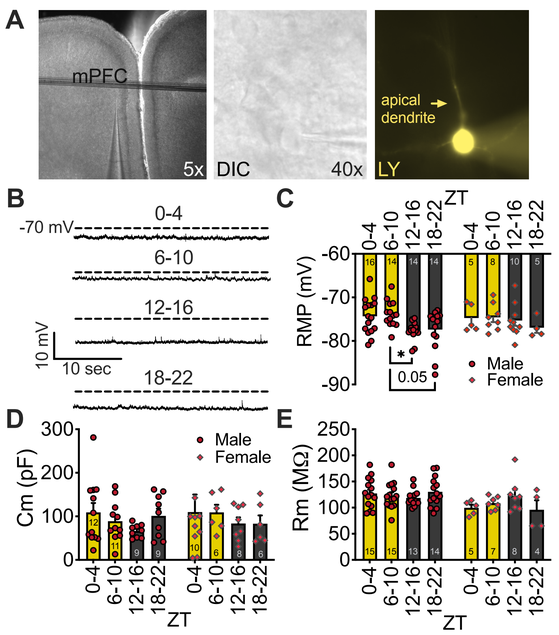

Figure 1. ***A,*** Image of mPFC slice (*left*) and layer 2/3 pyramidal neuron *(middle)* backfilled with lucifer yellow (LY, *right*). ***B,*** Representative traces of current clamp recordings at each ZT bin. ***C***, Mean membrane potential (RMP) for ZT bins 0-4, 6-10, 12-16, and 18-22 for male and female mice ***D,*** Mean membrane capacitance (Cm) and (**E**) resistance (Rm). *One-way ANOVA; n values inset on bars (error ± SEM) *p <0.05*

Excitatory inputs are time of day dependent and differ in male and female mice

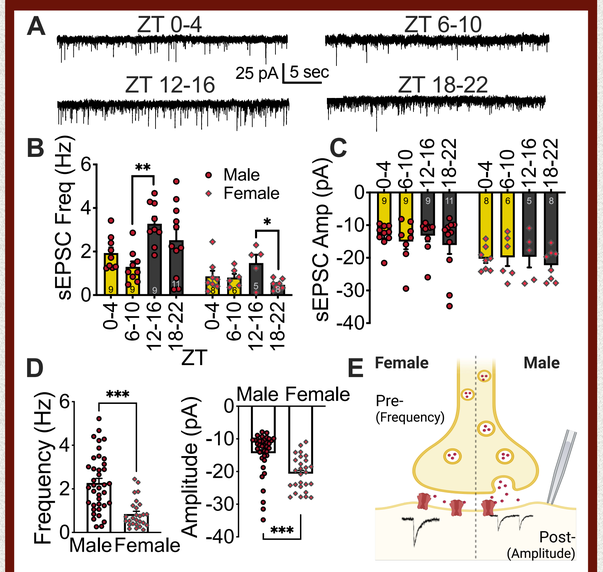

Figure 2. ***A***, Representative sEPSC traces at each ZT bin and ***B***, Mean sEPSC frequency and (**C**) amplitude in male and female mice. ***D,*** Comparison of all male and female sEPSC frequency (*left*) and amplitude (*right*). ***E,*** Diagram of sex differences in excitatory synaptic transmission. ***p <0.01, ***p<0.001.*

Throughput of inhibitory inputs are time of day dependent and differ with by sex

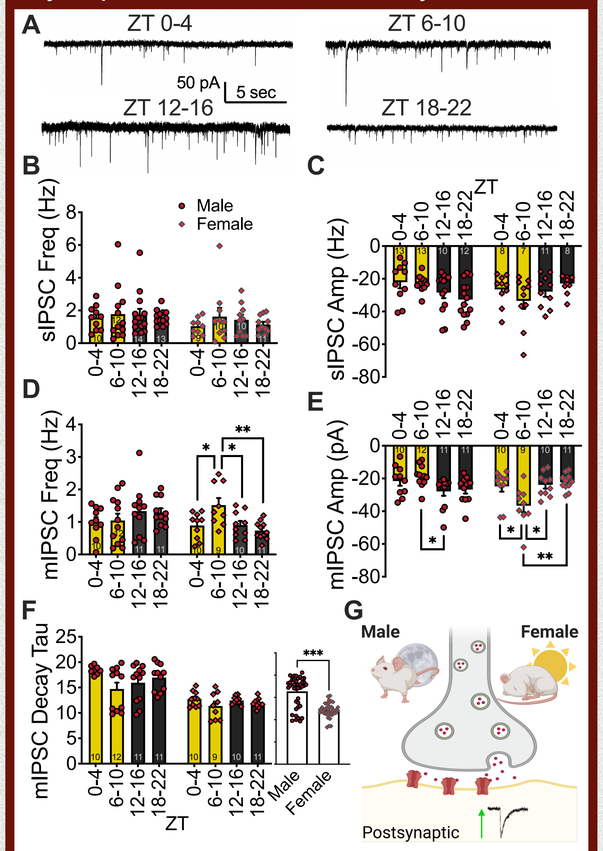

Figure 3. ***A***, Representative sIPSC traces at each ZT bin and ***B***, Mean sIPSC frequency and (**C**) amplitude in male and female mice. **D,** Mean mIPSC frequency (**E**) amplitude and (**F**) decay tau with between sex comparison (*right*). **G.** Diagram of sex differences in time of day changes in inhibitory synaptic transmission. **p <0.05, **p<0.01.*

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SUPPORT

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Time of day alters action potential firing threshold and decay tau in male mice

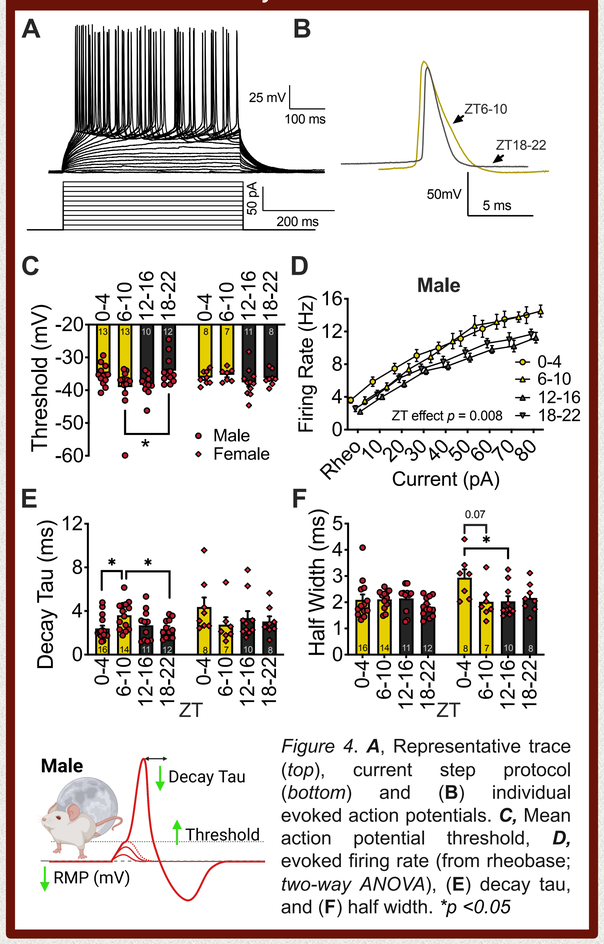


Figure 4. ***A***, Representative trace (*top*), current step protocol (*bottom*) and (**B**) individual evoked action potentials. ***C,*** Mean action potential threshold, ***D,*** evoked firing rate (from rheobase; *two-way ANOVA*), (**E**) decay tau, and (**F**) half width. **p <0.05*

Inward and outward anionic currents are rhythmic in male, but not female mice

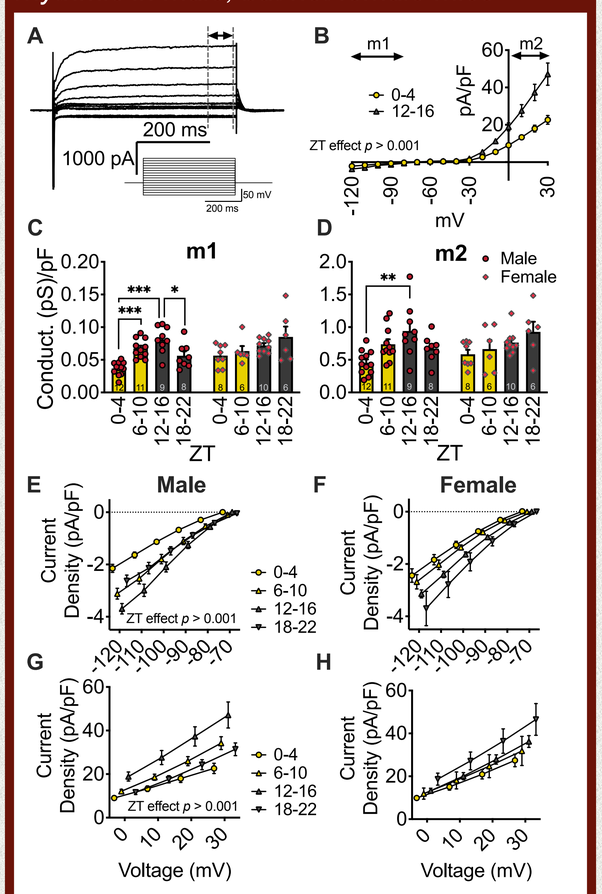


Figure 5. ***A***, Representative trace (*top; dashed line represents delayed current averaged for analysis*) and voltage step protocol (*bottom*). ***B,*** current-voltage (IV) relationship at ZT 0-4 and 12-16 in male mice (*normalized to cell capacitance*). ***C,*** Mean conductance calculated from inward (-120 to -80mV) and (**D**) outward (0 to 30mV) current. ***E,F,*** Inward and (**G,H**) outward current density at each ZT bin in male *(left)* and female *(right)* mice. **p <0.05*

Daily rhythms of pyramidal neuron conductance is K⁺ channel dependent

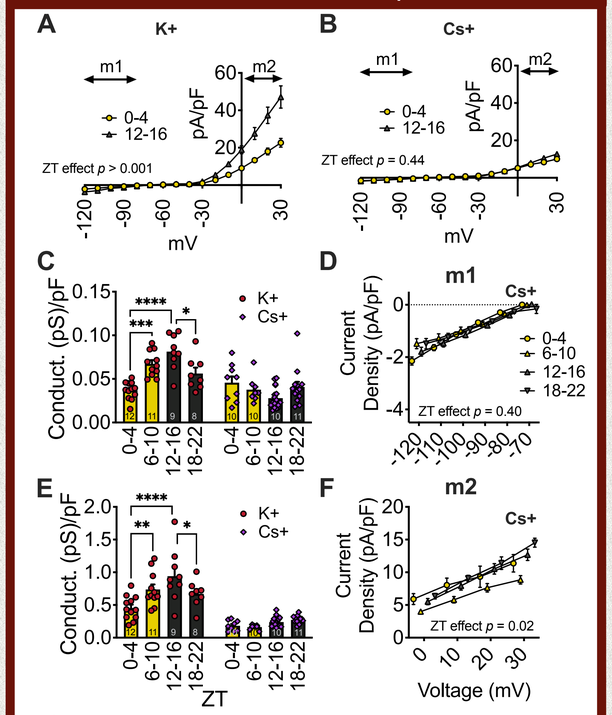


Figure 6. ***A***, IV relationship at ZT 0-4 and 12-16 in male mice (*normalized to cell capacitance*) with a K-gluconate or (**B**) CsCl internal solution. ***C,*** Mean conductance calculated from (**D**) inward (-120 to -80mV) and (**E,F**) outward (0 to 30mV) current slope using K^+ (red circles) or Cs^+ (purple diamond) internal solution. **p<0.05, **p<0.01.*

SUMMARY

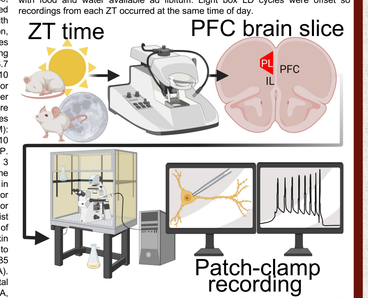
Here we show that in the prelimbic PFC:

- Pyramidal neurons are hyperpolarized during the active period in male mice
- Excitatory inputs increase during the dark period
- Females have less excitatory inputs, but stronger throughput
- Strength of inhibitory inputs changes with time of day and are opposite in males and females
- Information throughput has a higher threshold during the subjective day in males
- Potassium channel activity regulates daily rhythms in the physiology of pyramidal neurons

METHODS

Electrophysiology. Mice were euthenized 1h prior to their ZT bin (i.e., at ZT23 for recording bin ZT0-4). Brains were immediately removed and the forebrain was blocked in a 0-4◦C NMDG cutting solution (mM): 92 NMDG, 2.5 KCl, 1.25 NaH2PO4, 30 NaHCO3, 3 sodium pyruvate, 2 thiourea, 20 HEPES, 10 MgSO4, 0.5 CaCl2, 25 glucose, 20 sucrose, pH 7.4 with HCl. Forebrains were mounted adjacent to each other and sectioned simultaneously with a sapphire knife (Delaware Diamond Knives, Wilmington, DE) yielding ~3 mPFC slices (250-µm) per mouse. Slices were allowed to recover for 30 min at RT in recording artificial cerebrospinal fluid (aCSF) (mM): 124 NaCl, 3.7 KCl, 2.6 NaH2PO4, 26 NaHCO3, 2 CaCl2, 2 MgSO4, 10 glucose, and bubbled using 95% 02/5% C02. For recording, slices were transferred to a perfusion chamber containing aCSF maintained at 34-37°C. Neurons were visualized with a Zeiss Axoskop 2. Recording electrodes were back-filled with internal solutions as follows (mM): Current-clamp and sEPSCs; 125 K-gluconate, 10 KCl, 10 NaCl, 5 HEPES, 10 EGTA, 3 NaATP and 0.25 NaGTP. sIPSCs; 140 CsCl, 5 MgCl2, 1 BAPTA, 10 HEPES, 3 NaATP, and 0.25 NaGTP. EPSCs were recorded in the presence of bicuculline (30 µM). sIPSCs were recorded in the presence the competitive AMPA/kainate receptor antagonist CNQX (10 µM), the selective NMDA receptor antagonist APV (50 µM), and the glycine antagonist strychnine (2 µM). Miniature (m) IPSCs had the addition of the voltage-gated sodium channel blocker tetrodotoxin (TTX; 1 µM). Patch electrodes (3-5MΩ) were guided to neurons with an MPC-200-ROE controller and MP285 mechanical manipulator (Sutter Instruments, Novato, CA). Recordings were collected with a UPC-10 USB dual digital amplifier and Patchmaster NEXT software (HEKA, Holliston, MA). Only neurons with holding currents not exceeding 100pA at V_H= -70mV for a 10-min control period (input resistance > 70 MΩ), a series resistance < 50MΩ, and drift <10% were studied further. Reagents were obtained from Tocris Cookson, Cayman Chemical, and Sigma Aldrich.

Mice. Animal procedures and experiments were approved by the University of Massachusetts Amherst IACUC in accordance with the U.S. Public Health Service Policy and NIH Guide for the Care and Use of Laboratory Animals. Male and female 8-16 week old wild-type mice (Charles River) on a C57BL/6J background were group-housed in light boxes at 25°C, under a 12/12-hr light/dark (LD) cycle, with food and water available ad libitum. Light box LD cycles were offset so recordings from each ZT occurred at the same time of day.



Statistics. Data was analyzed in Patchmaster NEXT or converted with ABF Utility (Synaptosoft) for analysis in Clampfit (Molecular Devices). Datasets were tested for normality before statistical analysis. Comparison of ZT effects was made using one-way ANOVA or Kruskal-Wallas test for datasets that failed normality. Two-way ANOVA was used for comparison of the IV relationships. Statistics were calculated with Prism 9 (Graphpad).