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NEURAL ACTIVITY IN THE SUPRACHIASMATIC CIRCADIAN CLOCK OF NOCTURNAL MICE ANTICIPATING A DAYTIME MEAL

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Abstract—Circadian rhythms in mammals are regulated by a system of circadian oscillators that includes a light-entrainable pacemaker in the suprachiasmatic nucleus (SCN) and food-entrainable oscillators (FEOs) elsewhere in the brain and body. In nocturnal rodents, the SCN promotes sleep in the day and wake at night, while FEOs promote an active state in anticipation of a predictable daily meal. For nocturnal animals to anticipate a daytime meal, wake-promoting signals from FEOs must compete with sleep-promoting signals from the SCN pacemaker. One hypothesis is that FEOs impose a daily rhythm of inhibition on SCN output that is timed to permit the expression of activity prior to a daytime meal. This hypothesis predicts that SCN activity should decrease prior to the onset of anticipatory activity and remain suppressed through the scheduled mealtime. To assess the hypothesis, neural activity in the SCN of mice anticipating a 4–5-h daily meal in the light period was measured using FOS immunohistochemistry and *in vivo* multiple unit electrophysiology. SCN FOS, quantified by optical density, was significantly reduced at the expected mealtime in food-anticipating mice with access to a running disk, compared to *ad libitum*-fed and acutely fasted controls. Group differences were not significant when FOS was quantified by other methods, or in mice without running disks. SCN electrical activity was markedly decreased during locomotion in some mice but increased in others. Changes in either direction were concurrent with locomotion, were not specific to food anticipation, and were not sustained during longer pauses. Reduced FOS indicates a net suppression of SCN activity that may depend on the intensity or duration of locomotion. The timing of changes in SCN activity relative to locomotion suggests that any effect of FEOs on SCN output is mediated indirectly, by feedback from neural or systemic correlates of locomotion.

Key words: suprachiasmatic nucleus, circadian rhythms, food entrainment, food anticipation, multiple unit activity, FOS.

INTRODUCTION

Circadian activity rhythms in rodents are regulated by a light-entrainable circadian pacemaker in the suprachiasmatic nucleus (SCN), and by food-entrainable circadian oscillators (FEOs) located elsewhere in the brain and/or body. When food availability is unrestricted, nocturnal rodents are active and eat primarily at night, under control of the SCN pacemaker. If food is restricted to the middle of the day, when nocturnal rodents normally sleep, a daily rhythm of food anticipatory activity (FAA) emerges. The rhythm has canonical properties of circadian clock control, but does not require the SCN pacemaker (Boulos and Terman, 1980; Mistlberger, 1994; Stephan, 2002). The location of FEOs hypothesized to mediate FAA remains uncertain, although candidate sites include the hypothalamus, cerebellum and striatum (Sutton et al., 2008; Mendoza et al., 2010; Mistlberger, 2011; Gallardo et al., 2014).

In nocturnal rodents, the SCN pacemaker actively promotes sleep and suppresses activity in the light period and does the opposite in the dark period (Mistlberger, 2005; Fleshner et al., 2011). The SCN is comprised of a population of coupled circadian clock cells which, in aggregate, generate an approximately sinusoidal daily rhythm of multiple unit electrical activity (MUA) with a peak at mid-day and a trough at mid-night (Houben et al., 2014). FEOs responsible for FAA are presumed to generate a similar daily rhythm, but synchronized to mealtime rather than light-dark (LD). For meals scheduled in the light period, this creates a conflict between SCN and FEO output. FEOs have been hypothesized to resolve this conflict in two ways, by directly activating arousal circuits and by suppressing SCN output prior to mealtime (Mistlberger, 2006; Moriya et al., 2009; Acosta-Galvan et al., 2011; Landry et al., 2011).

If FEOs directly impose a daily rhythm of inhibition on SCN output, then SCN neural activity should decrease prior to the onset of FAA, and remain suppressed through the end of the expected mealtime, spanning pauses in anticipatory or consummatory behavior. This hypothesis

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Abbreviations: AVP, arginine vasopressin; FAA, food anticipatory activity; FEOs, food-entrainable oscillators; LD, light-dark; MUA, multiple unit electrical activity; NGS, Normal Goat Serum; OD, optical density; PBS, phosphate-buffered saline; SCN, suprachiasmatic nucleus; ZT, Zeitgeber Time.

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is supported indirectly by evidence that in nocturnal rodents fed *ad libitum*, SCN MUA (Meijer et al., 1997; Yamazaki et al., 1998; Schaap and Meijer, 2001; Van Oosterhout et al., 2012) and FOS expression (a molecular marker of neural activity) (Janik and Mrosovsky, 1992; Antle and Mistlberger, 2000) are reduced by locomotor activity in the light period. However, suppression of SCN MUA, when evident, appears concurrent with or after the onset of activity. It has therefore been interpreted as activity-dependent, rather than predictive of, or causal to activity (van Oosterhout et al., 2012). The suppression of SCN FOS following stimulated activity or arousal has also been interpreted as a response to behavior, mediated by inhibitory inputs to the SCN from the intergeniculate leaflet (NPY, GABA) and midbrain raphe (5HT, GABA), both of which are activated when animals are aroused and moving (Janik and Mrosovsky, 1992; Grossman et al., 2000; Saderi et al., 2013; Webb et al., 2014).

Few studies have recorded SCN electrical activity during restricted feeding schedules, and no quantitative data are available on changes in SCN activity prior to mealtime (Inouye, 1982). At least 10 studies have quantified SCN FOS expression in rats, mice or Syrian hamsters anticipating a mid-day meal, with four reporting a decrease (Challet et al., 1997; Acosta-Galvan et al., 2011; Blum et al., 2012b; Escobar et al., 2007), three an increase (Nakahara et al., 2004; Girotti et al., 2009; Mitra et al., 2011) and three no change (Angeles-Castellanos et al., 2004; Begriche et al., 2012; Dantas-Ferreira et al., 2015). Regardless of the direction of any change, FOS is an indirect measure of neural activity integrated over time. Without sampling at multiple time points during the hours immediately preceding mealtime, analysis of FOS cannot reveal whether changes in SCN neural activity precede or follow the onset of FAA, or whether SCN activity recovers during pauses in FAA.

To obtain more direct evidence by which to evaluate the hypothesis that anticipation of a daytime meal involves inhibition of SCN activity, we made electrical recordings from the SCN of freely moving mice with food available *ad libitum* and then restricted to a 4-h daily meal in the light period. To gain insights into the variability of results from previous studies of SCN FOS, we also assessed FOS expression immunohistochemically, using three different quantification methods and two recording conditions (i.e., with and without a running disk).

EXPERIMENTAL PROCEDURES

Subjects and housing

Experiments to quantify the immediate early gene product FOS in the SCN were conducted at Simon Fraser University (Burnaby, Canada) and were approved by the University Animal Care Committee. Male C57BL/6J mice (N = 37, Charles River PQ) received at 45 days of age were single-housed in standard plastic mouse cages equipped with wire mesh food hoppers, corn cob bedding, and a plastic Igloo house (11 cm diameter, 5.7 cm tall; Igloo Fast-Trac, BioServ, Flemington, NJ, USA) with or without a running disk (depending on group assignment) mounted horizontally at a shallow angle on the top of the Igloo. Running disk rotations were recorded continuously using a commercial interface and data acquisition system (Clocklab, Actimetrics, USA). Passive infrared motion sensors were mounted above each cage to continuously record movement. Water was available *ad libitum*. Cages were housed in cabinets in a climate controlled vivarium (22 ± 2 °C, ~50% humidity), with a 12:12 LD cycle (12 h of ~70 lux light provided by white LEDs).

Experiments to record electrical activity from multiple SCN neurons were conducted at the Leiden University Medical Center (Leiden, The Netherlands) in accordance with the regulations of Dutch law on animal welfare and were approved by the institutional ethics committee for animal procedures. Male C57Bl/6J mice were housed in a controlled environment (22 °C, 40–50% humidity) in LD 12:12. Following surgical procedures (described below) the mice were housed in standard type III cages (425 × 266 mm) with stainless steel wire lids and a hinged divider. The cage was enriched with a 1-cm layer of sawdust bedding (Woody Clean S 8/15, Rettenmaier, Germany) and paper tissues (no running disk).

Feeding schedules

Mice used for quantification of FOS immunoreactivity were acclimated to the recording cages for 12 days with food available *ad libitum* (rodent chow 5001, BioServ, USA). The mice were then assigned randomly to one of three groups. Mice in the restricted feeding group (N = 13) were food deprived for 14 h and then provided food 7 h after lights-on (Zeitgeber Time (ZT) seven, where ZT0 is lights-on by convention) for 28 days. The duration of food access was gradually decreased from 10 h to 5 h over the first 2 weeks, and maintained at 5 h for the remainder of the schedule. Mice in the acute food deprivation group (N = 13) were provided food *ad libitum* for 28 days and were then food deprived for 20 h, beginning at ZT12 (lights-off). Mice in the food *ad libitum* group (N = 11) were not food deprived. Five mice in each group were maintained without running disks throughout the experiment. After day 28 of restricted feeding, mice in all three groups were euthanized and perfused for brain extraction at ZT8 (mice in the food restriction and acute food deprivation groups were not fed on that day).

Mice used for recording SCN MUA were provided food (rodent chow RM3, Special Diet Services, Sussex, UK) and water *ad libitum* prior to restricted feeding. Food was then removed for 28 h, and provided at ZT7 for 4 h daily for 4–24 days. In two cases, the mice were maintained in constant dark (DD) during the last 2 days of restricted feeding.

In vivo electrophysiological SCN recordings

For SCN electrode implantation, mice were anesthetized using a mixture of Ketamine (100 mg/kg, Aescoket, Boxtel, The Netherlands), Xylazine (10 mg/kg, Bayer AG, Leverkusen, Germany) and atropine (0.1 mg/kg, Pharmachemie, Haarlem, The Netherlands) and mounted in a stereotactic device (Digital Just for Mouse