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PHASE PREFERENCE FOR THE DISPLAY OF ACTIVITY IS ASSOCIATED WITH THE PHASE OF EXTRA-SUPRACHIASMATIC NUCLEUS OSCILLATORS WITHIN AND BETWEEN SPECIES

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Abstract—Many features of the suprachiasmatic nucleus (SCN) are the same in diurnal and nocturnal animals, suggesting that differences in phase preference are determined by mechanisms downstream from the SCN. Here, we examined this hypothesis by characterizing rhythmic expression of Period 1 (PER1) and Period 2 (PER2) in several extra-SCN areas in the brains of a diurnal murid rodent, *Arvicanthis niloticus* (grass rats). In the shell of the nucleus accumbens, dorsal striatum, piriform cortex, and CA1 of the hippocampus, both PER1 and PER2 were rhythmic, with peak expression occurring at ZT10. PER1 in the dentate gyrus also peaked at ZT10, but PER2 was arrhythmic in this region. In general, these patterns are 180° out of phase with those reported for nocturnal species. In a second study, we examined inter-individual differences in the multioscillator system of grass rats. Here, we housed grass rats in cages with running wheels, under which conditions some individuals spontaneously adopt a day active (DA) and others a night active (NA) phase preference. In the majority of the extra-SCN regions sampled, the patterns of PER1 and PER2 expression of NA grass rats resembled those of nocturnal species, while those of DA grass rats were similar to the ones seen in grass without access to running wheels. In contrast, the rhythmic expression of both PER proteins was identical in the SCN and ventral subparaventricular zone (vSPZ) of DA and NA animals. Differences in the phase of oscillators downstream from the SCN, and perhaps the vSPZ, appear to determine the phase preference of particular species, as well as that of members of a diurnal species that show voluntary phase reversals. The latter observation has important implications for the understanding of health problems associated with human shift work.

Key words: suprachiasmatic nucleus, extra-SCN oscillators, *Arvicanthis niloticus*, night active, day active, human shift work.

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Abbreviations: Acc-shell, accumbens shell; BLA, basolateral amygdala; BNST-ov, oval nucleus of the bed nucleus of stria terminalis; CEA, central amygdala; DA, day active; DG, dentate gyrus; DSt, dorsal striatum; LD, light–dark cycle; NA, night active; PC, piriform cortex; PER1, Period 1; PER2, Period 2; SCN, suprachiasmatic nucleus; vSPZ, ventral subparaventricular zone; ZT, Zeitgeber time.

Circadian rhythms in physiological, metabolic, and behavioral functions are endogenous, and when entrained to the light–dark cycle, they enable organisms to anticipate daily environmental challenges. In mammals, the suprachiasmatic nucleus (SCN) of the hypothalamus is the primary circadian pacemaker, which is necessary for the maintenance of a multitude of rhythms, as well as for their entrainment to the day–night cycle (Moore and Eichler, 1972; Stephan and Zucker, 1972; Ralph et al., 1990; Klein et al., 1991; Dibner et al., 2010). At the cellular level, the SCN generates rhythms using molecular mechanisms of transcriptional and translational feedback loops involving sets of clock genes (Welsh et al., 1995, 2010; Reppert and Weaver, 2001; Ko and Takahashi, 2006). Most of what we know about the functioning of the mammalian SCN comes from studies using nocturnal laboratory species, but consistent with the pioneer work of Schwartz and colleagues (Schwartz et al., 1983), there is a growing literature showing that some fundamental features of the SCN are common to species with widely varying activity patterns (see Smale et al., 2008 for a review). Most importantly, the coupling of rhythms in expression of circadian clock genes in the SCN to the light–dark cycle are very similar in day- and night-active species [reviewed in (Smale et al., 2008)], as are rhythms in expression of genes that are part of SCN output pathways (Dardente et al., 2004; Lambert et al., 2005; Mahoney et al., 2009). These observations have lead to the suggestion that differences in the circadian systems of diurnal and nocturnal species reside downstream from the SCN circadian pacemaker (Kalsbeek et al., 2008; Smale et al., 2008).

The molecular clock machinery first described in the SCN is also present in several brain regions and peripheral tissues (Mendoza and Challet, 2009; Dibner et al., 2010). These extra-SCN oscillators may regulate rhythms in region specific functions (Hastings et al., 2008; Mendoza and Challet, 2009). Studies with nocturnal laboratory rodents have reported rhythmic expression of the clock genes *Per1, Per2, Bmal1, Clock*, and *Cry*, and their protein products in extra-SCN brain regions (Abe et al., 2002; Shieh, 2003; Guilding and Piggins, 2007; Feillet et al., 2008; Amir and Stewart, 2009b). These extra-SCN oscillators need circadian signals from the SCN to entrain to the light/dark cycle (Yamazaki et al., 2000; Abe et al., 2002; Amir et al., 2004; Lamont et al., 2005; Guilding et al., 2009). Very little information is available on clock gene expression in the extra-SCN oscillators of diurnal animals (Mrosovsky et al., 2001; Vosko et al., 2009). We recently reported that there are PER1 and PER2 rhythms in the amygdala and the oval

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nucleus of the bed nucleus of the stria terminalis (BNST-ov) of the diurnal grass rat (*Arvicanthis niloticus*) (Ramanathan et al., 2008b, 2010). In those regions, PER1 and 2 expression peaks during the light phase of the light-dark cycle, approximately 180° out of phase with the peaks reported for the same brain regions in nocturnal species (Amir et al., 2004; Lamont et al., 2005; Angeles-Castellanos et al., 2007; Feillet et al., 2008). These results are consistent with the hypothesis that a phase reversal of extra-SCN oscillators contributes to differences in phase preference of nocturnal and diurnal species (see also Lambert and Weaver, 2006).

In addition to light, the phase of several extra-SCN oscillators can be modulated by a variety of non-photic cues that alter activity patterns of animals. These include, for example, meal schedules (Wakamatsu et al., 2001; Angeles-Castellanos et al., 2007; Feillet et al., 2008; Verwey and Amir, 2009), chronic exposure of methamphetamine in the drinking water (Masubuchi et al., 2000; Iijima et al., 2002), cocaine sensitization (Abarca et al., 2002; Uz et al., 2002; Manev and Uz, 2006), and hormonal rhythms (von Gall et al., 2002; Amir et al., 2004; Lamont et al., 2005; Amir and Stewart, 2009a). One non-photic cue that has a major impact on the activity rhythms of grass rats is access to a running wheel. Although these animals are fundamentally diurnal in the laboratory and in the field (Katona and Smale, 1997; McElhinny et al., 1997; Blanchong and Smale, 2000), when given free access to running wheels, a subset of them switches to a predominantly night active (NA) pattern, while the rest continues to be day active (DA) (Blanchong et al., 1999; See Fig. 1). This switch takes place without any apparent changes in the SCN, or in the region just above the nucleus, the ventral subparaventricular zone (vSPZ); for example patterns of Fos expression in the SCN and vSPZ are unaffected as the grass rats become predominantly nocturnal (Rose et al., 1999; Mahoney et al., 2000, 2001; Smale et al., 2001; Schwartz and Smale, 2005). This does not appear to be the case for rhythms in other brain regions. For example, rhythms in Fos expression in orexin-producing neurons of the perifornical lateral hypothalamus are completely reversed in DA and NA grass rats (Nixon and Smale, 2004). Thus, engaging in activity during the normal resting period could change the phase of extra-SCN brain oscillators, without affecting those of the SCN or vSPZ, which could disrupt the temporal organization of the organism. Such changes in the internal temporal program may contribute to the many health problems associated with human shift work. Animals that voluntarily shift their activity to their resting phase provide an excellent model to study the physiological and behavioral consequences of individual differences in phase preference for the display of activity in humans (e.g., Gau et al., 2007).

Here we further document that species can be quite different with respect to the phase of extra-SCN oscillators (Experiment 1), and we evaluate the hypothesis that the phases of many extra-SCN oscillators relative to the light–dark (LD) cycle are more malleable than are those of the SCN and the vSPZ (Experiment 2). In Experiment 1 we monitored rhythms of PER1 and PER2 expression in the dorsal striatum (DSt), piriform cortex (PC), shell of the nucleus accumbens (Acc-shell), dentate gyrus (DG) and area CA1 of the hippocampus and compared them to those reported for nocturnal rodents. In Experiment 2, we looked at whether voluntary changes in phase preference for the display of activity are accompanied by changes in phase in oscillators outside the SCN and vSPZ. Thus, Experiment 2 tested the hypothesis that features that accompany phase differences in the display of activity between species, also accompany chronotype differences within a species.

EXPERIMENTAL PROCEDURES

Animals and housing

Adult male grass rats from our breeding colony at Michigan State University, East Lansing, MI, USA, were housed individually in Plexiglas cages (34×28×17 cm^3), under a 12:12-h LD cycle, with a red light (<5 lx) on all the time, and with free access to food (PMI Nutrition Prolab RMH 2000, Brentwood, MO, USA) and water. For Experiment 2 (see below), the animals were housed in the same Plexiglas cages, but now equipped with running wheels (26 cm diameter; 8 cm width). The colony room was entered at irregular intervals to check the health of the animals and to replenish food and water. All experiments were performed in compliance with guidelines established by the Michigan State University All University Committee on Animal Use and Care, and the National Institute of Health guide for the Care and Use of Laboratory Animals.

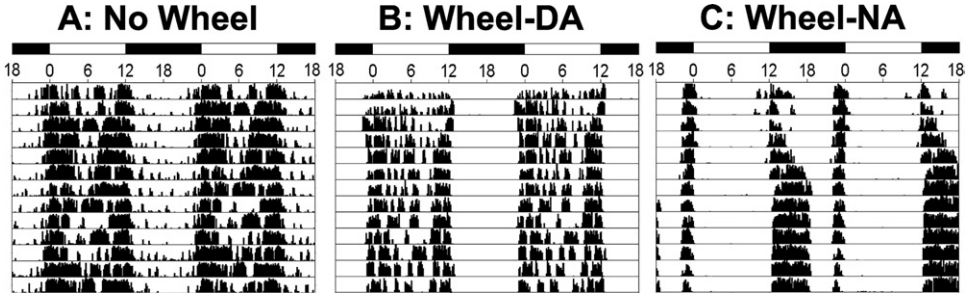

Fig. 1. Double-plotted actograms depicting the activity patterns of grass rats from our colony when they are housed without access to running wheels (panel A), or with access to running wheels and showing either a day-active (DA) chronotype (panel B) or a night-active (NA) chronotype (panel C). The dark bars at the top represent the 12-h dark periods and the numbers below the bars refer to Zeitgeber times.