Locomotor Exercise and Circadian Rhythms in Mammals

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Abstract

The timing of locomotor activity is a major rhythmic output of the mammalian circadian system but both spontaneous and induced exercise can also themselves impact on circadian rhythms across levels of organisation and in multiple tissues, including both the brain and periphery. This review briefly summarises historical research on the influences of locomotor activity and its correlates on circadian function and then discusses recent advances in this area. Locomotor exercise can acutely alter circadian phase, both of overt behavioural rhythms and in peripheral tissues, and can stably entrain rhythms in behaviour driven by the hypothalamic master circadian pacemaker in the suprachiasmatic nucleus (SCN). Locomotor activity potently and acutely suppresses both electrical activity and clock gene expression in the master circadian pacemaker of the hypothalamus, though mechanistically how this is achieved is still unclear, as are the effects of locomotor activity in diurnal species, including humans.

Highlights

The timing of locomotor activity is a major output of the mammalian circadian system.

Locomotor exercise also impacts on circadian function, a so-called Zeitnehmer.

Locomotor activity suppresses electrical firing and clock gene expression in the SCN

Locomotor-circadian influences are poorly studied in diurnal species.

Scheduled activity may stabilises aberrant circadian function.

Introduction

The vast majority of organisms on earth have evolved in a highly rhythmic environment, dominated by predictable daily changes related to the solar day, including cycles of light and darkness, predator and prey activity, and temperature. Endogenous daily, or circadian, timing systems have evolved to allow organisms to anticipate time in this cyclic environment, enabling them to sequester their behavioural and physiological activities to appropriate temporal niches [1]. In animals, these circadian timing systems consist of networks of endogenous biological clocks contained within most cells and tissues throughout the body; in mammals these networks of circadian clocks are arranged in a hierarchical structure with a 'master' circadian pacemaker located in the suprachiasmatic nucleus (SCN) of the hypothalamus, and tissue specific peripheral oscillators located throughout the rest of the body that function to control local aspects of physiology [2].

To be of value to an organism its circadian system must be synchronised with environmental time. This is achieved in part by light signals, detected by the retina and delivered directly to the SCN, but also by 'non-photic' time signals which are principally based on indulgence in, or induction of, locomotor activity and/or arousal [3]. It is now widely recognised that locomotor activity and arousal can act as time signals, or Zeitgebers, for endogenous circadian clocks, with locomotor activity itself representing both an input signal to circadian systems and a robustly rhythmic clock-controlled output, a so-called Zeitnehmer [4].

Locomotor Activity, Arousal and Circadian Rhythms

The profound ability of locomotor exercise to impact on circadian rhythms is well illustrated by the observation that simply providing a laboratory rodent with unrestricted access to a home-cage running-wheel is sufficient to significantly alter the free-running period of the animal's circadian system [5] (Fig. 1). Characterising this interaction further, a variety of different techniques have been used to acutely induce locomotor activity in nocturnal laboratory rodents with the aim of manipulating

circadian function. These include presentation with, or confinement to, a novel running wheel, forced treadmill activity, presentation with the opportunity to run voluntarily in a home-cage running wheel, brief (a few hours) pulses of darkness, and injection with benzodiazepines [6, 7]. Using these approaches, locomotor activity has been shown to interact with and manipulate circadian rhythms in a number of ways; free-running rhythms can be entrained by scheduled opportunities to engage in voluntary locomotor exercise, even showing anticipatory activity prior to wheel availability [8-10]; re-entrainment of behavioural rhythms to a shift of the light-dark cycle can be dramatically hastened by locomotor activity induced when a nocturnal animal is confined to a novel running wheel [11, 12], or by injection with benzodiazepines [13]; and acute phase shifts in behaviour can be induced by the same stimuli [14] (Fig. 1). Importantly, the magnitude of the effects of locomotor activity on behavioural rhythms is dose dependent, correlating well with the amount of locomotor activity involved, and similarly, when locomotor activity in a running-wheel is prevented during the above procedures the expected effects on behavioural rhythms are either strongly attenuated or abolished altogether [6, 15]. Interestingly, other manipulations that alter the total amount of locomotor activity, for example activation of oestrogen receptors, also impact on the circadian profile of activity and attenuate responses to light pulses, a common feature of locomotor-related non-photic stimuli [16]. Given the close association of locomotor activity and behavioural arousal it is also appropriate to note here that the induction of arousal, sometimes in the absence of induced locomotor activity, can also engage the circadian system, presumably via common mechanisms and exemplified by the use of gentle handling to deprive sleep during the rest phase [17].

Whilst some differences are seen between nocturnal species, the effects of locomotor activity and induction of arousal on circadian behaviour consistently generate a characteristic non-photic phase-response curve (PRC) profile with large phase-advances during the middle of the subjective day, extending slightly into the early subjective night, and a smaller phase-delay zone during the mid-late subjective night and early subjective day. Moreover, the phase-angles of entrainment of nocturnal

animals to scheduled opportunities to exercise are also broadly consistent with this non-photic PRC [9, 18, 19] (Fig. 1).

Diurnal Mammals, Circadian Clocks and Locomotor Activity

Similarities and differences between the circadian systems of nocturnal and diurnal species have long been an under-researched but commonly debated subject in chronobiology. Whilst some aspects of SCN anatomy, neurochemistry and physiology are similar between nocturnal and diurnal species, including the timing of fundamental circadian activities at the cellular and molecular levels and responses to photic stimuli, the nature of critical differences that allow organisms to occupy entirely different photic and temporal niches remain to be clearly identified [20]. With respect to the effects of locomotor activity on nocturnal and diurnal circadian systems, the situation is similarly unresolved. The vast majority of research on the actions of locomotor activity to alter circadian function has been conducted using nocturnal species, with only relatively few investigations of diurnal animals. This issue is further confounded by complex temporal activity profiles in some species. For instance, the degu (*Octodon degus*), which can readily switch between different activity patterns depending on environmental conditions and the presence of a homecage running wheel [21], and recent reports of environmental niche switching in some primarily nocturnal species in response to environmental and metabolic factors, including the induction of locomotor activity [22, 23]. Nonetheless, locomotor activity is certainly an effective Zeitgeber in diurnal species. Daily opportunities to exercise can entrain or modulate free-running rhythms in the exclusively diurnal European ground squirrel (*Spermophilus citellus*) [24] and diurnal/crepuscular degu [25]. However, the profile of non-photic PRCs for primarily diurnal species remains unclear; the phase-angle of entrainment for both ground squirrel and degu rhythms to scheduled exercise is consistent with a nocturnal-like non-photic PRC [24, 25], though injections of downstream neurochemical correlates of non-photic stimuli and arousal by gentle handling phase shift behavioural rhythms in *Arvicanthis* grass rats with a different phase-response profile [26-28]. It should, however, be noted that both of these investigations of locomotor activity

effect *per se*, in the ground squirrel and degu, yield the expected non-photic PRC profile, while the apparently contradictory reports of differing profiles for *Arvicanthis* are elicited using either general mild arousal due to handling and sleep deprivation, or injections of chemicals assumed to act downstream of locomotor activity/arousal, rather than the manipulation of behavioural locomotor activity itself.

Effects of Locomotor Activity on the SCN

Locomotor activity and related signals adjust the phase of overt circadian rhythms in behaviour by altering endogenous rhythms generated by the master circadian pacemaker in the SCN. Rhythms in this structure, and in peripheral circadian oscillators, manifest from the inter-linked expression of a set of core clock genes including the *period*, or *per*, and *cryptochrome*, or *cry*, genes [29]. In the SCN of nocturnal animals, this system is ultimately associated with the generation of higher electrical activity during the subjective day and lower at night [30]. Within the SCN, locomotor activity [31] and manipulation of related downstream neurochemical correlates [32-34] act to potently suppress the expression of the *per* genes in nocturnal species (Fig. 2), while suppression of *per* mRNA using antisense oligonucleotides during the subjective day leads to non-photic-like phase shifts in behaviour [35]. Effects in diurnal species, however, remain somewhat unclear [26].

Similarly, locomotor activity also potently, and in this case rapidly and acutely, suppresses electrical activity in the SCN (Fig. 2) in a dose dependent manner [36], thus increasing the amplitude of SCN rhythms in electrical activity, and presumably also clock gene expression [37, 38]. Intriguingly, scheduled locomotor exercise during the early part of the night reduces the amplitude of SCN PER2::LUC rhythms monitored *ex vivo* [39], though a recent report of simultaneous real-time monitoring of two core clock genes in the SCN of freely moving rats may soon allow the monitoring the both acute and long term effects of locomotor exercise on SCN clock gene expression *in vivo* with hitherto unachievable temporal and molecular resolution [40]. Moreover, it is currently unclear how

locomotor exercise affects specific cell populations within the SCN, which may not always conform to the tissue-level average output rhythm of electrical activity [41], though further technological advancements may be needed before this can be addressed.

The suppressive effects of locomotor exercise on SCN electrical activity are consistent with the primarily suppressive actions in the SCN of neuropeptide Y and GABA, as well as serotonin, the main neurochemicals understood to signal locomotor activity effects to the SCN from the intergeniculate leaflet (IGL) of the visual thalamus, and the raphe nuclei of the brain stem, respectively (extensively reviewed in [6]). In addition to these, other neurochemicals and pathways also appear to be important for mediating the effects of locomotor activity and arousal on the SCN, including enkephalins and neurotensin from the IGL, orexins from the lateral hypothalamus [15, 42], and cholinergic innervation of the SCN from the basal forebrain, which appears to be necessary for arousal-mediated phase shifts in behaviour [43]. Moreover, the effects of locomotor exercise on neural activity in the brain are not limited to the SCN, with exercise acutely reducing cortical activity [44], among a host of other actions [45].

Effects of Locomotor Activity on Peripheral Circadian Clocks

Scheduled exercise under a light-dark cycle can alter the phase of physiological parameters such as heart rate and body temperature in nocturnal laboratory rodents, and both unrestricted [46] and scheduled exercise [39, 47-49] also adjust the phase of clock gene expression in the adrenal glands, skeletal muscle and liver (Fig. 2), as well as period and amplitude in some peripheral tissues. Induction of locomotor activity by exposure to a novel running wheel hastens re-entrainment of lung and skeletal muscle circadian clocks to an advance in the phase of the light-dark cycle [11]. Together, these reports suggest that manipulating the timing of locomotor exercise offers a valuable intervention to address the problems with internal circadian desynchrony commonly associated with shift-work and

jet lag [50]. Further, mistimed running has been reported to interfere with re-entrainment of clock gene rhythms in muscle and lung [51], indicating that care should be taken to identify the correct circadian phase for locomotor exercise to be used in this manner. Similar investigations in diurnal species will also be needed in order to realise any true translational potential of these reports. It should also be noted that alterations to the timing of peripheral oscillators may be indirectly affected by other behavioural and physiological correlates of locomotor activity [52].

Locomotor Activity, Arousal and Stress

The physiological correlates of locomotor exercise that mediate circadian responses of peripheral oscillators and SCN-driven behavioural rhythms have yet to be fully determined. Recently, both glucocorticoids and signalling via sympathetic pathways have been implicated as contributing to entrainment of peripheral oscillators. Adrenalectomy and injection of a adrenergic antagonists attenuate phase-shifts of peripheral oscillators to forced scheduled exercise under a light-dark cycle [48]. Interestingly, in this paradigm, whilst voluntary exercise was also reported to induce peripheral phase shifts, these were smaller than those seen in response to forced exercise, and only forced exercise induced higher serum levels of corticosterone and norepinephrine. This suggests that stress pathways may, under some circumstances, potentiate peripheral phase shifts in response to exercise [18, 48], and is consistent with an earlier report of stress-induced phase adjustment of peripheral oscillators [53]. This potential role of stress in contributing to circadian alterations due to locomotor exercise is, however, unresolved as conflicting reports have suggested that induction of acute stress may act to inhibit the effects of locomotor activity on the manipulation of SCN-driven circadian rhythms in behaviour [15]. This issue will require further investigation but it is possible that this apparent contradiction may be related to either differential effects on the SCN and periphery, or the complexity of glucocorticoid expression profiles, involving oscillations on both ultradian and circadian timescales, as well as the induction of very high concentrations of glucocorticoids by acute stress events [54]. Acute changes in glucose availability, oxygen levels, free reactive oxygen species, pro-

and anti-inflammatory cytokine signalling, pH and temperature may all also be present in peripheral tissues during and following exercise. Each of these may be capable of interacting with core circadian processes at the molecular level via a number of different mechanisms and all remain possible candidates for involvement in mediating exercise-induced changes in circadian function [18].

Conclusions and Perspectives

It is clear, based on our current understanding of the interactions between locomotor exercise and circadian biology, that locomotor activity and its correlates can act as *bona fide* Zeitgebers for circadian oscillators. In animal models, locomotor activity can elicit both stable entrainment and acute manipulation of the circadian phase of behavioural rhythms (Fig. 1), as well as engaging with and altering key oscillator properties at tissue level (Fig. 2). Indeed, locomotor activity has also been reported to improve rhythms both in aged mice [38] and in mutant strains with disrupted SCN intercellular synchrony [8], whilst a lack of exercise has been reported to reduce scale invariance of behavioural rhythms, a marker of general health [55]. Further, the timing of exercise within a light-dark cycle has been shown to be important for the degree of protection offered by exercise against weight gain on a high fat diet [56], and simulated shift-work desynchronises liver gene expression, disrupting metabolism [47]. Intriguingly, recent work has also revealed a critical link between elements of the circadian core molecular machinery and physical performance; *cry1* and *cry2* appear to perform key roles in controlling energy storage and metabolism in skeletal muscle, ultimately limiting exercise capacity [57]. Within the SCN, beyond acute effects on gross extracellular firing rate, the effects of locomotor exercise on electrical activity remain to be determined, for instance effects on specific neurotransmitter activities are unknown, as are effect in specific sub-populations of neurons and in different neuroanatomical compartments. Similarly detailed information on the effects of locomotor exercise on clock gene expression in the SCN is also currently lacking.

In humans, a diversity of circadian-related impacts of physical exercise have been reported. Discrete bouts of exercise can induce acute phase shifts, though more work is needed to generate consensus on the PRC profile of such responses [58-60]. Timed exercise can also accelerate human re-entrainment to shifts of the light-dark cycle equivalent to transatlantic travel [61, 62]. During pregnancy, maternal exercise is reported to modify foetal circadian rhythms [63], whilst increased physical activity in elderly men is positively correlated with higher amplitude oscillations in expression of the *per3* gene, as measured in hair follicles [64]. As such, in cases of circadian disruption, which are otherwise associated with a remarkable variety of negative health outcomes, there is significant potential for locomotor activity to be employed as a non-invasive intervention to improve circadian integrity and general health.

Figure Legends

Figure 1. Behavioural Responses to Locomotor Exercise. (**A**) Providing nocturnal rodents with unrestricted access to a home cage running wheel (red shaded area) significantly shortens their free-running circadian period. (**B**) Induction of wheel-running at the new onset of darkness (red oval) hastens re-entrainment to a shift in the phase of the light-dark cycle. (**C**) Induced wheel-running (red ovals) yields large phase advances in behavioural rhythms during the mid-subjective day, and smaller phase advances during the late subjective night and early subjective day. These responses define a 'classical' non-photic phase response curve (PRC; **D**). Mice with a free-running period of less than 24h entrain to daily scheduled exercise (red boxes) by delay (**E**), while mice in a longer than 24h free-running period entrain by advance [9, 15, 19](**F**). The phase angles of entrainment of these entrainment responses to scheduled exercise are consistent with the non-photic PRC. In all panels black bars represent the main daily active phase and shaded areas indicate times of darkness.

Figure 2. Effects of locomotor exercise in SCN and Peripheral Oscillators. Locomotor activity acutely supresses *per* clock gene expression in the SCN, and acutely reduces SCN firing in real time *in vivo*. This effect on SCN electrical activity boosts the amplitude of daily variation in SCN action potential firing by further suppressing firing during the subjective night when rates are already low. This is likely also the case for the effects of locomotor activity on *per* expression, though this has yet to be empirically tested. Scheduled locomotor exercise in a running wheel or on a treadmill can phase shift oscillations in clock gene expression in peripheral tissue oscillators.

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Fig. 1

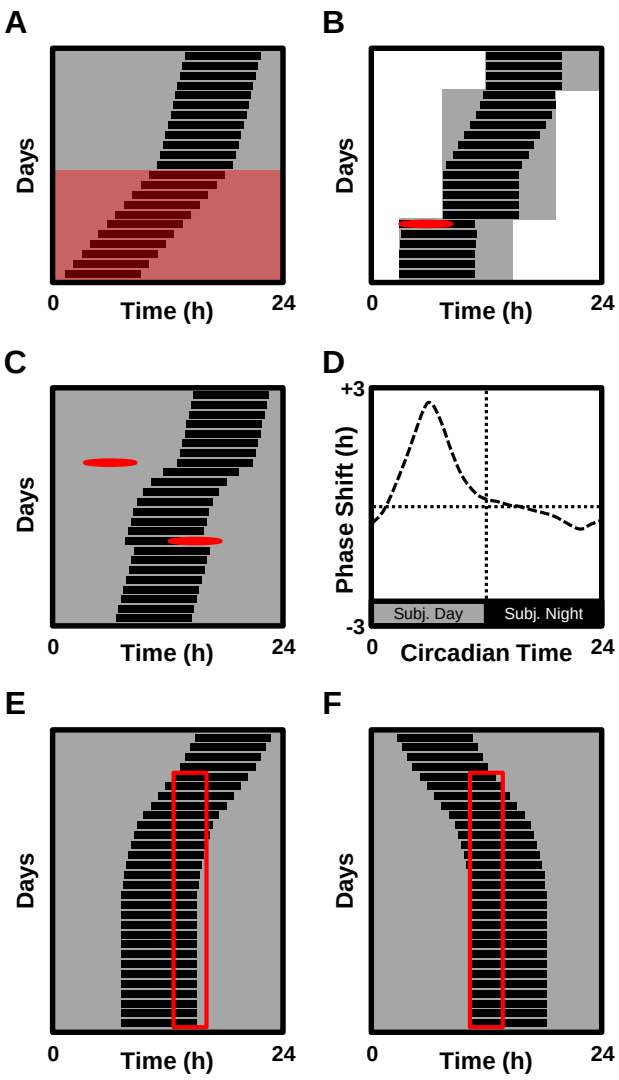
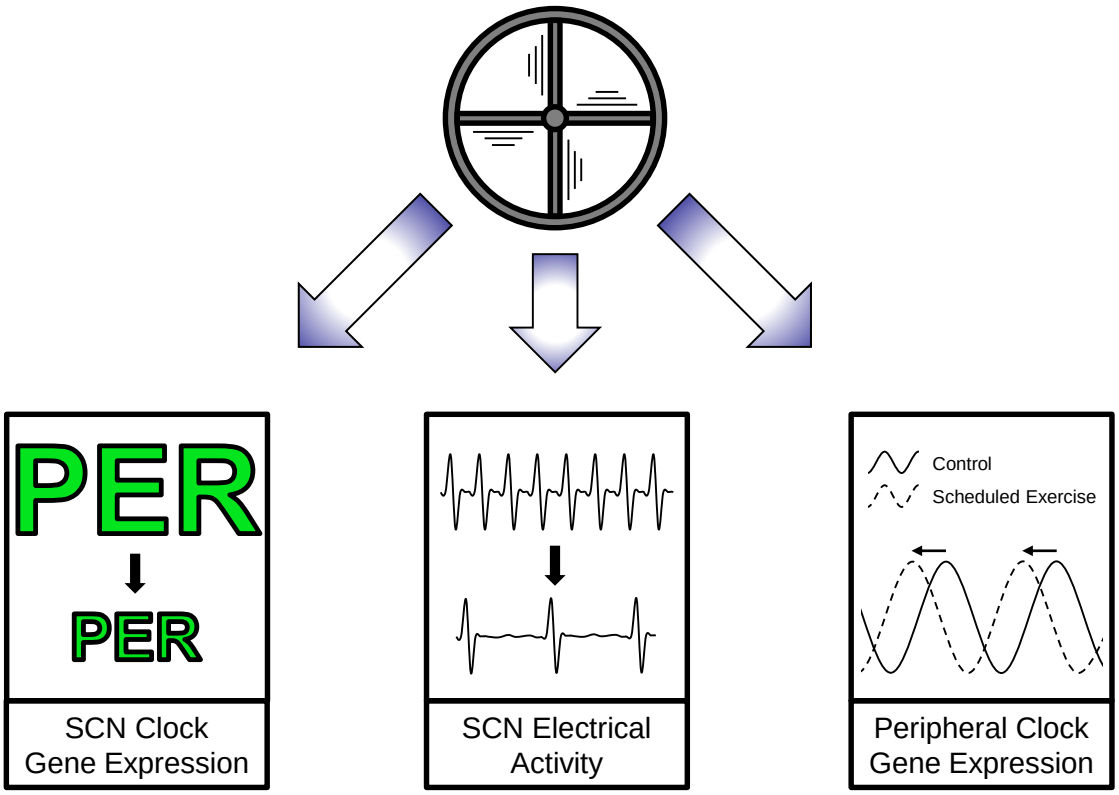

A
Days
0
Time (h)
24
B
Days
0
Time (h)
24
C
Days
0
Time (h)
24
D
+3
Phase Shift (h)
-3
Subj. Day
Subj. Night
0
Circadian Time
24
E
Days
0
Time (h)
24
F
Days
0
Time (h)
24

Fig. 2


PER
PER
SCN Clock Gene Expression
SCN Electrical Activity
Control
Scheduled Exercise
Peripheral Clock Gene Expression