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How does general anaesthesia affect the circadian clock?

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Short title: Anaesthesia affects the circadian clock

Summary (200 words)

Post-operative patients experience sleep disturbances. Animal studies demonstrate that general anaesthesia (GA) can disrupt circadian rhythms and cause changes in the molecular clock, indicating that anaesthesia contributes to post-operative circadian disruption. Here we review the effect of anaesthesia on the circadian clock and its rhythms in order to summarise current findings outline commonalities between studies and propose mechanisms by which effects may be mediated.

Key points: (1) GA has strong effects on the main neurotransmitter systems linked with circadian control (GABA/NMDA) and may act by interfering with light-entrainment of the clock. (2) Expression of the core clock gene *per2* is inhibited by GA (possibly via a NMDA/GSK3 β pathway). (3) GA's effect on circadian rhythms appears greatest when administered during animals' active phases (4) GA may have different effects when administered under free-running and entrained conditions. (5) Anaesthesia may mimic the mechanism involved in adaptation of the clock to changes in daylength.

There is agreement that GA can strongly affect the circadian clock. How anaesthesia-induced changes in the molecular clock lead to changes in behaviour remains unclear. The answer, and what it may mean for patients post-operatively, will rely on systematic studies at molecular, behavioural, and clinical levels using standardised protocols.

Keywords

GABA, NMDA, suprachiasmatic nucleus, sleep/wake cycle, clock genes, post-operative sleep disruption, Brain and muscle ARNT-like 1, zeitgeber, hypnotic drugs

Glossary of terms

Free-running period	The time required for one complete oscillation of the circadian clock in the absence of any external stimulus.

Peripheral clocks	Circadian clocks in tissues other than the suprachiasmatic nucleus.
Phase shift	An advance or delay in the cycling of the circadian clock or a circadian rhythm.
Zeitgeber	Literally *Time-giver,* the external stimulus which usually acts to keep the circadian clock entrained by phase shifting the endogenous circadian clock mechanism.

Abbreviations

ARNT	Aryl hydrocarbon receptor nuclear translocator
BMAL1	Brain and muscle ARNT-like 1
CLOCK	Circadian locomotor output kaput
CRY	Cryptochrome
GA	General anaesthesia
GABA	Gamma aminobutyric acid
GSK3β	Glycogen synthase kinase 3β
NMDA	N-methyl-D-aspartate
PER	Period
SCN	Suprachiasmatic nucleus
TTFL	Transcription/translation feedback loop

Despite the widespread use of general anaesthetics, their mode of action is poorly understood. Natural sleep is controlled by the combined actions of two different but related mechanisms, the sleep homeostat and the circadian clock (Reviewed in (1)). As general anaesthesia can induce a state of depressed consciousness at a time of day when these control mechanisms promote maximum wakefulness, it seems likely that anaesthetics act at least in part by usurping or overriding one or both of these pathways. Indeed anaesthetics have been shown to profoundly affect the sleep homeostat (2-4) and sleep debt can be recovered during prolonged periods of anaesthesia or sedation with certain general anaesthetic drugs. Quantifying the magnitude of these effects remains difficult because of widely varying protocols. There is now considerable interest in determining the effect of anaesthetics on the circadian clock.

For some time it has been known that the duration of anaesthesia elicited by a standard drug dose differs depending on the time of day at which an anaesthetic is administered, indicating that the circadian clock can influence anaesthetic action (reviewed in (5, 6)). More recently the reciprocal has also been found to be true: there are now more than 20 published studies demonstrating that anaesthetics can disrupt the circadian clock and alter circadian rhythms (7-27). The aim here is to review this literature, identifying key findings and proposing potential mechanisms. Throughout the review key points including commonalities between studies and proposed mechanisms are highlighted in italics.

In clinical practice, analgesics and muscle relaxants are used in combination with hypnotic anaesthetic drugs. Here we consider the hypnotic agents in isolation. Although a number of physiological and behavioural processes are under circadian control, the effects of anaesthesia on rest/activity rhythms has received considerably more research attention than the effects on other circadian outputs. In this review we focus on *in vivo* studies which have examined the influence of

anaesthesia on rest/activity rhythms, as well as both *ex vivo* and *in vitro* studies which have examined anaesthesia-induced effects on the molecular clock driving these rhythms.

Determining how anaesthesia affects the clock is not only of scientific interest but also holds potential clinical relevance. Patients frequently experience sleep disturbances post-operatively. Anaesthesia-induced clock disruption may be a contributing factor (28). Circadian disruption and associated sleep disturbances are linked with adverse effects on mood and cognitive performance as well as inflammation and immune function (29-31). Thus anaesthesia-induced circadian disruption may impede post-operative recovery on multiple levels.

The behaviour and physiology of most living things is attuned to the Earth's day/night cycle. This synchrony is accomplished in part by an endogenous circadian clock. Circadian clocks are present within most cells in the body where they provide temporal control of cell activity (32). In mammals a group of neurons located in the suprachiasmatic nucleus (SCN) of the hypothalamus house the "master" circadian clock. The SCN clock co-ordinates the activity of clocks in non-SCN tissue (peripheral clocks) thereby controlling circadian rhythms at the whole organism level (33). It is sensitive to external stimuli ("zeitgebers") and can be re-set by both naturally occurring cues (e.g. light) and artificial agents (e.g. pharmaceuticals) (34, 35).

At a molecular level, the circadian clock consists of four core components. In mammals these are the proteins BMAL1 (brain and muscle ARNT-like 1), CLOCK (circadian locomotor output kaput), PERIOD (PER1, PER2 and PER3 in humans) and CRYPTOCHROME (CRY1 and CRY2 in humans) (36-38). BMAL1 is a transcription factor which forms a dimer with CLOCK (37), a histone acetylase (39), to regulate gene transcription. BMAL1:CLOCK promote *per* and *cry* transcription but auto-inhibit *bmal1* expression (40, 41). PER and CRY also form a dimer and among other functions (40) act as an inhibitor of BMAL1:CLOCK transcriptional activity (41). Consequently mRNA and protein levels of

BMAL1 oscillate in antiphase with those of PER and CRY in a self-sustaining cycle (40, 41). The kinetics of the transcription/translation feedback loops (TTFL) involved are such that, in the absence of any external stimuli, one complete oscillation of the PER:CRY/BMAL1:CLOCK cycle is completed in approximately 24h. The exact period of one oscillation, the free-running period, is slightly less or slightly more than 24h, depending on inter-species and inter-individual variances, but is seldom exactly 24h. Exposure to the Earth's day/night cycle changes the kinetics of the TTFL so that the duration of one complete oscillation of the PER:CRY/BMAL1:CLOCK cycle exactly matches the Earth's 24h day/night cycle.

The molecular clocks in a subset of neurons located in the ventral core region of the SCN are directly entrained to light through innervation by the retinohypothalamic tract (42, 43). The retinohypothalamic tract is activated when light is detected by photoreceptors and specialised retinal ganglion cells containing the photopigment melanopsin within the eye (44). This results in activation of NMDA receptors on the SCN neurons (42), initiating an intracellular signalling cascade which ultimately leads to transcription of *per* (45). GABAergic signalling between retinohypothalamic-innervated neurons and other neurons in the SCN enables synchronisation within the SCN (46). Consequently, during the hours of light PER (and CRY) protein levels peak in the SCN whereas during the hours of darkness BMAL1 and CLOCK protein levels peak.

GABA also has a critical role in re-setting the clock in response to changes in the light/dark cycle (47, 48). Both $GABA_A$ and $GABA_B$ receptors are involved in mediating light-induced phase shifts in the clock (47). Exposure to light during the normal dark period triggers an increase in activation of GABA receptors in SCN neurons (47, 49). Sustained activation of GABA receptors phase shifts the molecular clock (49), can inhibit *per2* expression, and can lead to either phase advances or delays (50).

NMDA and GABA are therefore critical for regulating the circadian clock. This is particularly pertinent for understanding the effect of anaesthesia on the circadian clock as most anaesthetics are NMDA receptor antagonists and/or GABA agonists (51-57). However it should be noted that volatile anaesthetic drugs are atypical, with low potency, and bind promiscuously to at least ten percent of all the proteins in the body (58).

Part of the mechanism by which the SCN clock controls behaviour and physiology is through the regulation of neurotransmitter and hormone release as well as BMAL1-mediated gene transcription (59). Secretion of a number of hormones including melatonin, cortisol and insulin is regulated by the circadian clock (59, 60). Circulating hormone levels affect the phasing of clocks in peripheral tissues such as the liver and heart and hence provide circadian control of physiological processes (61).

General anaesthesia disrupts rest/activity rhythms in animal models

In humans, the post-operative and post-anaesthetic period is frequently associated with disrupted circadian rhythms (28, 62, 63). Although anaesthesia is believed to contribute to this disruption (28), hospitalisation, surgery and disease are known to profoundly influence circadian rhythms (e.g. the lack of cortisol circadian rhythms recorded in ICU patients) (64, 65), and therefore confound results. Since it is generally considered unethical to administer anaesthesia to humans unless required medically, animal models have proven particularly valuable in this field.

Social and lifestyle cues often mask circadian behaviour in humans, however, in the laboratory setting daily rest/activity cycles of other animals can faithfully represent clock phase. As a result, monitoring activity patterns in animals is a useful proxy for monitoring the clock. Animals maintained in a light/dark cycle (LD 12:12) demonstrate a clear rest/activity pattern due to the light-entrained clock. In contrast, the period of the rest/activity cycle in animals maintained under constant conditions (e.g. constant darkness) is representative of the free-running period of the SCN

(66). Exposure to zeitgebers will phase shift the SCN and this will result in a phase shift in the rest/activity cycle (66). Results from animal studies (summarised in Table 1) clearly demonstrate that anaesthetics can phase shift and disrupt behavioural circadian rhythms. This effect is dependent on a number of factors as discussed below.

Anaesthesia during the active period of an animal is more frequently associated with disrupted rest/activity rhythms than anaesthesia during the rest period.

Disruption to rest/activity rhythms may manifest as increased activity during the normal rest period for an animal or decreased activity during the normal active period. To date, four studies have examined the effect of anaesthesia (sevoflurane, ketamine, pentobarbital and isoflurane) during the normal active phase of an animal on subsequent activity levels. All four described disrupted rest/activity rhythms as a result of anaesthesia, with three showing a decrease in locomotor activity during the following active phase (8, 13, 15) and one showing an increase in activity during the subsequent rest phase (17).

The effects of anaesthesia administered during the rest phase of an animal is less clear. No change in activity level was observed in studies in which ether, ketamine or pentobarbital was administered during the rest period (13, 18), however, total activity was reduced following sevoflurane treatment (8, 15). This may be due to differing effects of these drugs on the sleep homeostat. Some anaesthetics (eg. propofol (3)) are known to lead to a reduction in sleep debt during periods of anaesthesia whereas sleep debt continues to accrue during anaesthesia with other agents such as sevoflurane unless anaesthesia is very deep (3). It seems likely that anaesthesia during the rest period with agents which reduce sleep debt will have minimal effects on subsequent rest/activity rhythms as they are capable of mimicking at least to some extent the effects of natural sleep.

Disruption of rest/activity rhythms may also manifest as a change in the phase of the rest/activity cycle. To date, 11 studies have been published which have examined the effect of anaesthesia (between one and eight hours duration) on the phase of the rest/activity cycle (7, 8, 10-13, 15, 17-20). Figure 1 summarises the phase change reported in each of these studies with respect to the approximate time of anaesthesia relative to activity onset. The value of this approach is to enable direct comparison of results from studies involving nocturnal and diurnal animals. Despite differences in experimental protocols some general patterns emerge.

[Insert figure one here]

Figure 1 Effect of the type of anaesthetic drug and the time of administration on rest/activity rhythms. Summary of results from all published studies describing an effect of anaesthesia on the phase of the rest/activity cycle. The magnitude and direction of the phase shift reported in each study is shown with respect to the approximate time at which anaesthesia was administered relative to activity onset. *Data has been pooled from multiple studies which have utilised different experimental protocols and different animal models.*

In the majority of studies administration of anaesthesia (pentobarbital, sevoflurane or isoflurane) during the animal's *inactive period* had no effect on the phase of locomotor activity rhythms (7, 8, 10, 12, 13) or resulted in relatively small phase delays (of between 15-25 mins) (7, 11, 15) which, if acute, are unlikely to be of physiological relevance. The notable exceptions are ketamine, which induced a phase delay of 3.9h when administered at the mid-point of the inactive period in rats (13), and propofol, which induced a phase delay of approximately 1h when administered during the latter half of the inactive period in mice (19, 20)

In contrast, all except one study involving pentobarbital (13), demonstrated that anaesthesia during the *active period* leads to phase shifts in the rest/activity cycle (7, 10-13). There is considerable variation between studies in terms of the magnitude and direction of the shifts elicited. One possible source of this variability may be that different types of anaesthetic drugs have different effects on rest/activity rhythms. In support of this, Mihara *et. al.* found ketamine-induced anaesthesia resulted in a large phase advance when administered during the active phase, and a large phase delay when administered during the rest phase. A similar duration of pentobarbital had no effect on phase at either timepoint (13). Unlike pentobarbital (and many other anaesthetics), ketamine is not a GABA agonist (51-54); and produces a state of anaesthesia that is qualitatively different and is termed "dissociative anaesthesia". Ketamine has a plethora of molecular targets. Apart from NMDA blockade it increases brain amines and acetylcholine, and induces widespread modulation of gene expression. It is important to be cautious when comparing the effects of two such different anaesthetic agents, not only because of their different molecular targets but also because they are cleared differently from the body and thus may be acting for different durations of time. This point may also be illustrated by the study of Prudian *et. al*. which found that animals anesthetised for 50 mins with ketamine (which requires hepatic clearance) were significantly less active for the remainder of the day compared to animals anesthetised for the same duration with ether (18). This suggests that the recovery time following ketamine administration was longer; likely a reflection of its slower clearance. Ketamine is known to produce significant clinical analgesia, anti-depression, and hallucinations at concentrations one tenth of those required for anaesthesia (67). It is possible that these low concentrations may still exert a significant phase shifting effect on rest/activity rhythms. If so we could expect that, when it is used as a drug of abuse, the resultant circadian disruption and sleep deficit might contribute significantly to the prolonged psychiatric disturbances associated with its abuse.

The effect of anaesthesia on rest/activity rhythms may differ in light-entrained compared to free-running conditions

Although mechanistic differences between various anaesthetics may contribute to the variability in their effects on rest/activity rhythms, differences in the experimental protocols between studies is a further confounding factor and may be one of the biggest causes of the variability in results. Figure 2 summarises results from studies examining the phase shifting effect of anaesthesia administered during the *active period* grouped depending on whether they were conducted under entrained or free-running conditions. It appears that irrespective of the type of anaesthetic used, experiments conducted in free-running conditions are likely to cause phase delays whereas in entrained conditions phase advances may be more common.

[insert figure 2 here]

Figure 2 Phase shifts in rest/activity rhythms observed following anaesthesia during the active period in free-running compared to light-entrained animals. The data for free-running conditions (orange) are from two studies involving honeybees (in one study anaesthesia was administered at multiple time points during the active period). The data for entrained conditions (pink) and "entrained, switched to free-running at/near time of anaesthesia" (yellow) is from studies involving nocturnal rodents. *Different anaesthetic agents and different experimental protocols have been used in each study (refer Table 1).*

Interestingly, in two studies mice entrained to a light/dark cycle and then transferred to constant darkness (either on the day of anaesthesia or a few days before) (11, 15), displayed very small phase delays even when the drug was administered during the animal's active phase. These studies are the only ones addressing the effects of sevoflurane on the phase of the rest/activity cycle. The apparent lack of an effect of sevoflurane may be attributable not only to differences in the action of

sevoflurane compared to other anaesthetics but also to the protocol, which may have mitigated the phase-shifting effect of sevoflurane. As the free-running period of these mice is shorter than 24hrs (68), the transfer of animals from light-cycles into constant conditions will result in a daily advance in the onset of their activity. If anaesthesia treatment of free-running animals results in phase delays (as Figure 2 suggests), then the apparent lack of effect of sevoflurane in these studies may be due to two opposing forces acting on the clock simultaneously.

General anaesthesia may inhibit light-entrainment of the circadian clock

From a mechanistic perspective, it is plausible that anaesthetic agents could interfere with light-entrainment of the circadian clock. As the retinohypothalamic tract is NMDA receptor dependent (69), anaesthetic agents such as isoflurane, sevoflurane and ketamine, which are powerful NMDA receptor antagonists (55-57), could be expected to inhibit retinohypothalamic signalling and therefore light entrainment of the clock. Sustained activation of GABA receptors can also phase shift the clock and inhibit light-induced phase advances in rhythms (47, 49). Prolonged anaesthesia with GABA agonists (isoflurane, sevoflurane, propofol and pentobarbital) may therefore also interfere with light entrainment of the clock.

In support of an interaction between anaesthesia and light, Ludin *et. al*. observed that whilst bright light could phase advance rest/activity rhythms in bees and isoflurane could cause phase delays, concomitant exposure to bright light and isoflurane had no phase-shifting effect (7). These results not only indicate that anaesthesia can prevent light-induced phase advances in rest/activity rhythms and therefore interfere with light-entrainment of the clock, but also that light can prevent the phase-delaying effects of isoflurane. This latter observation may prove useful for combatting anaesthesia-induced sleep disruption in patients. The effect of drugs on light-entrainment of the clock is not without precedent as several muscle relaxants and sedatives have also been shown to prevent light-induced phase shifts in circadian rhythms (70, 71).

The effect of anaesthesia on rest/activity rhythms may differ between nocturnal and diurnal animals

A perennial problem with animal studies is that the most commonly used mammalian model is nocturnal. The possibility that anaesthesia has a different effect on rest/activity rhythms in nocturnal vs. diurnal animals also needs to be explored, as most of the studies involving light-entrained animals have been conducted in nocturnal rodents (12, 13, 19). Given that nocturnal animals are active when BMAL1/CLOCK levels are high, whereas diurnal animals are active when PER/CRY is high, if anaesthesia influences behaviour by acting on the molecular clock it might be expected that the effects of anaesthesia would differ between the two. In support of this contention, injection of the GABA agonist muscimol directly into the SCN has been shown to cause phase advances in activity of nocturnal rodents (72) but phase delays in diurnal rodents (34). Anaesthetics which are GABA agonists may therefore also have opposing effects on nocturnal versus diurnal animals. We predict that anaesthesia of diurnal animals, such as humans, with propofol (a relatively pure GABA agonist) should cause a phase delay; which is in keeping with the common patient experience of lack of passage of time during anaesthesia.

General anaesthetic agents alter expression of components of the molecular circadian clock

There is clear evidence from both *in vivo* and *in vitro* studies that general anaesthesia, directly or indirectly, alters the expression of core clock components in the SCN and can phase shift the clock (8, 10, 11, 15, 16, 21, 73).

Anaesthesia may transiently inhibit <u>per</u> transcription causing phase shifts in the <u>per</u> expression cycle

Changes in *per* expression have consistently been observed in studies which have examined the effects of anaesthesia on the molecular clock (8, 10, 11, 15, 16, 21, 73). However this effect may be dependent on the circadian time of administration. Kadota *et. al.* found that *per2* mRNA levels were lower than controls in murine SCNs following *in vivo* exposure to sevoflurane only when anaesthesia was administered during the rising phase of the *per2* expression cycle (11). Four other studies also describe a reduction in *per2* expression following *in vivo* sevoflurane (8, 15, 73), dexmedetomidine or propofol treatment (21). In all cases the onset of anaesthesia was during the rising phase of *per2* expression. The possibility of phase shifts in *per2* expression was not examined in any of these studies. However Cheeseman *et. al.* and Xia *et. al.* observed phase delays in *per-m* and *per2* mRNA cycles, in bees and mice respectively, following isoflurane treatment (10, 16). In both of these studies, treatment occurred during or immediately preceding the rising phase of *per* expression and *per* mRNA levels were notably lower immediately following anaesthesia. Taken together, results from these studies suggest that anaesthetics such as isoflurane and sevoflurane can transiently suppress *per* expression and that this can lead to a phase delay in *per* cycling.

That an effect of anaesthesia on *per2* expression may only occur when anaesthesia is administered during the rising phase of the *per2* expression cycle suggests the mechanism involves inhibition of transcription, as an effect on *per2* transcript stability would not be restricted to just one phase of the expression cycle. I*n vitro* studies have demonstrated that sevoflurane treatment of cultured SCNs or of a neuronal cell line results in a reduction in *per2* promoter activity (8, 22, 23). In SCNs, this inhibition resulted in a phase delay in *per2* expression (8, 23). Levels of acetylated histone 4 (an epigenetic modification usually associated with active gene transcription (74)) were found to be significantly lower in the *per2* promoter from SCNs isolated from sevoflurane-treated mice (14) suggesting *per2* transcription is also repressed *in vivo*.

Anaesthetics could act via the promotion of proteosomal degradation of BMAL1

Although most studies have focussed on the effect of sevoflurane or isoflurane on *per2* mRNA, Bellet *et. al.* found that ketamine inhibited *per1* expression *in vitro* by preventing binding of the CLOCK:BMAL1 complex to the *per1* promoter (9). Given that CLOCK has histone acetyltransferase activity and acetylates both histones 3 and 4 (39), prevention of CLOCK:BMAL1 binding to the *per1* promoter is likely to lead to a reduction in levels of acetylated histones in the promoter region. Therefore ketamine-mediated inhibition of *per1* transcription may be due to a similar mechanism as sevoflurane-mediated inhibition of *per2*. This suggests that both of these anaesthetics inhibit CLOCK:BMAL1 promoter binding; reducing expression of *per1* and *per2*.

The repressive effect of ketamine on *per1* expression is abolished by inhibition of glycogen synthase kinase 3 β (GSK3 β) (9). GSK3 β directly phosphorylates BMAL1 thereby targeting it for proteasomal degradation (75). Therefore ketamine may promote degradation of BMAL1 and this reduction in BMAL1 levels may be responsible for the observed reduction in CLOCK:BMAL1 occupancy of the *per1* promoter. Treatment with isoflurane or short-term exposure to sevoflurane also leads to GSK3 β activation (76, 77) whereas treatment with propofol, pentobarbital or long-term exposure to sevoflurane inhibit GSK3 β activation (76, 78, 79). Unlike propofol and pentobarbital, ketamine, isoflurane and sevoflurane are NMDA receptor antagonists. As inhibition of NMDA receptors has been found to activate GSK3 β (80), this may be the pathway involved in the inhibitory effect of these anaesthetics on GSK3 β. We speculate that anaesthetics which are NMDA receptor antagonists promote degradation of BMAL1 protein by activating GSK3 β.

If BMAL1 levels are reduced following anaesthesia, it follows that mRNA levels of other clock components would also be altered. To date, only two studies have examined the effect of anaesthesia on expression of other core components of the clock. Phase delays occurred in *cry-m* in honeybees and *cry1* in SCNs of mice following isoflurane treatment (10, 16). No effect on expression of *clock* was observed in honeybees (10) but phase-advances in expression of both *clock* and *bmal1*

were recorded in the SCNs of isoflurane-treated mice (16). This phase advance was marked by an initial increase in *bmal1* and *clock* mRNA levels immediately following anaesthesia.

The phase advance in the expression of *clock* is interesting given that relatively little is known about the regulation of *clock* in SCNs. Whilst a circadian pattern of *clock* expression is common in peripheral tissues, expression of *clock* is believed to be constitutive in mammalian SCNs (81). If anaesthesia does alter *clock* expression, this would provide novel insight into our understanding of how the SCN circadian clock functions.

A concomitant phase advance in *bmal1* expression and phase delay in *per2* and *cry1* expression fits with the hypothesis that anaesthetics may promote proteasomal degradation of BMAL1 protein. Alternatively, a reduction in CLOCK protein levels, or reduced nuclear localisation of BMAL1 or CLOCK, could also explain these results. As BMAL1:CLOCK drives transcription of *per* and *cry* but inhibits *bmal1* transcription (40, 41), decreased BMAL1/CLOCK protein levels or decreased nuclear translocation of BMAL1/CLOCK would be expected to inhibit *per2* and *cry1* transcription but lead to prolonged *bmal1* transcription, potentially leading to phase shifts. Future studies which examine the effect of anaesthesia on both protein and mRNA levels of clock components are likely to be informative in establishing the mechanism by which anaesthesia affects the clock.

Anaesthesia-induced clock disruption may parallel the mechanism involved in compensation of the clock for changes in day-length

There have been a number of recent developments in the understanding of how the SCN clock compensates for seasonal changes in day-length. It is possible that anaesthesia-induced phase shifts in the SCN clock involve similar mechanisms.

It is generally accepted that the phase of the molecular clock can differ between neurons located in the dorsal shell of the SCN compared to the ventral core (48, 82). This phase difference emerges in response to a change in the light/dark cycle, such as can occur with trans-meridian travel or with the changing seasons (48, 82). A subset of neurons within the ventral core (likely those directly innervated by the retinohypothalamic tract) appear to rapidly synchronise to the new light/dark cycle whereas neurons in the dorsal region continue cycling (at least transiently) with the original light/dark cycle (82, 83). The resultant phase difference between clocks in the two SCN regions is largely attributed to GABAergic repulsive coupling (48). A recent study found that peripheral clocks tended to be synchronised with the phase of the clock within dorsal shell neurons (84). This suggests that whilst the ventral core of the SCN is important for receiving light-entraining cues (43), the dorsal shell may be important for governing downstream physiological and behavioural rhythms. Studies investigating the mechanism involved in light-induced phase shifts of the clock suggest that it is the emergence of a transient phase difference between the dorsal and ventral SCN which may be responsible for the symptoms of "jet lag" (82).

Clinical general anaesthetics are predominantly GABAergic drugs. It is an attractive hypothesis that the jet-lag-like symptoms experienced by patients are due to an anaesthesia-induced phase difference between clocks in the dorsal and ventral SCN regions. This is supported by Ohe *et. al*. who observed that *mPer2* RNA was localised almost exclusively in the dorsomedial shell of the SCN in sevoflurane-exposed mice, whereas expression was much more diffuse in non-anaesthetised animals (15). However Matsuo *et. al*. found no difference in the regional localisation of *per2* in SCNs treated with sevoflurane *in vitro* compared to controls (45). The lack of effect in this latter study may be a consequence of the use of isolated SCNs. SCNs cultured *in vitro* receive no retinohypothalamic input. Therefore NMDA receptors on retinohypothalamic-innervated neurons in SCNs cultured in vitro would presumably be inactive regardless of whether an anaesthetic drug was administered or not.

If general anaesthesia does result in a phase shift between the dorsal and ventral regions of the SCN, it would be expected that animals entrained to a short photoperiod would be particularly susceptible to a phase-delaying effect of general anaesthesia. Although most studies examining the effect of anaesthesia on entrained animals have used a LD 12:12 cycle, mice were initially entrained to an LD 14:10 cycle before transfer into constant darkness immediately prior to the day of anaesthesia (11, 15). Since these mice are nocturnal, animals were effectively entrained to a short active period in these studies. This may explain why small phase shifts in locomotor activity were observed when anaesthesia was administered during the rest phase in these studies (11, 15) but not in several others (7, 8, 10). It would be interesting to investigate the effect of day-length on sensitivity to the phase-shifting effects of anaesthesia, and also to determine if the jet-lag-like symptoms experienced by patients following general anaesthesia are more severe following treatment in the winter months compared to summer.

The link between the disruption of the molecular clock and anaesthesia-induced changes in rest/activity rhythms

The causal link between the effects of anaesthesia on the molecular clock and the effects of anaesthesia on the rest/activity cycle has yet to be established. Results from molecular studies show that anaesthetics can affect expression of the core clock components. Phase changes in melatonin secretion and body temperature rhythms have also been observed following anaesthesia (13, 24, 33, 85) providing some support for the notion that the change in molecular components of the clock translates into a change in circadian rhythms of physiology and behaviour. However, although melatonin secretion and body temperature rhythms are controlled by the SCN, both are also regulated by the adrenergic pathway (86). Many anaesthetics also act on adrenergic pathways (87, 88) and a change in melatonin secretion or body temperature cannot necessarily be attributed to an

effect of anaesthesia on the clock. Similarly, anaesthetics also affect the sleep homeostat which also regulates rest/activity rhythms (2). It cannot be assumed that the effect of anaesthetics on rest/activity rhythms is due to their influence on the molecular circadian clock alone.

To date studies examining the effect of anaesthesia on the molecular clock have largely been conducted in isolation from those examining the effect on rest/activity rhythms. As a result, it is difficult to correlate findings of the two. The major issue is that studies which have examined the consequences of anaesthesia on rest/activity rhythms have mainly involved nocturnal animals. The key finding of this work is that phase shifts are more likely if anaesthesia is administered during the animal's active phase. Although most of the studies of the effects of anaesthesia on the molecular clock have also been conducted in nocturnal animals, they have involved anaesthesia administered during the rising phase of the *per2* expression cycle; a time which corresponds to the inactive phase for a nocturnal animal. In order to understand the relevance of circadian clock disruption to anaesthesia-induced changes in behavioural rhythms, the alignment between molecular and behavioural studies needs to be improved.

Conclusions, implications and predictions

General anaesthesia during an animal's active phase causes disruption to rest/activity rhythms. The type and extent of disruption probably depends on the type of anaesthetic drug, the duration of anaesthesia and the presence of other clock-resetting stimuli such as a light/dark cycle. In contrast, administration of anaesthesia during the animal's rest phase appears less likely to cause disruption to rest/activity rhythms. Again this may be dependent on the type of anaesthetic drug used or whether anaesthesia is administered during the early or late part of the inactive phase. Since clinically general anaesthesia is usually performed during the day (active period), it would seem likely that anaesthesia may contribute to post-operative circadian disruption in humans. Phase shifts in the rest/activity cycle have been observed in light-entrained animals providing further support for

a detrimental effect of anaesthesia in humans. This indicates that the presence of a light/dark cycle does not necessarily override the phase shifting effect of anaesthesia. The study of the extent of sleep and circadian disruption caused by anaesthesia, surgery and hospitalisation in a controlled patient population is a priority. Compared to the effect of anaesthesia on rest/activity rhythms, the effect of anaesthesia on physiological rhythms has received much less research attention. It is possible that anaesthesia has different effects on different physiological processes and this needs to be determined in order to fully understand the impact of general anaesthesia in the clinical setting.

Although there is good evidence that anaesthesia alters the expression of components of the molecular clock, the mechanism by which this occurs has yet to be established. Given that GABA and NMDA regulate the central clock and are also targets of many anaesthetics, determining the role of these neurotransmitters in mediating anaesthesia-induced changes in the molecular clock and behavioural rhythms seems a logical next step. Based on our current understanding of the role of NMDA receptors and GABAergic signalling in the entrainment of the SCN clock, we propose the following hypotheses:

- *Anaesthetics which are NMDA receptor antagonists inhibit light entrainment of the SCN clock*
    - NMDA receptor antagonism mimics the effects of exposure to a prolonged period of darkness during the normal light period.
        - Anaesthetics like ketamine, nitrous oxide, and xenon, which are NMDA receptor antagonists (but not GABA agonists) may only induce phase shifts in animals exposed to a light/dark cycle and not in animals maintained in constant darkness.

- *Anaesthetics which are GABA agonists induce phase shifts in the circadian clock through sustained activation of GABA receptors.*

- Anaesthetics which are GABA agonists may induce phase shifts in animals maintained in constant darkness as well as in light-entrained animals.
- The duration of anaesthesia exposure may be important in determining whether phase-shifts occur as sustained activation of GABA receptors may be required.

Undoubtedly, the mechanisms involved in anaesthesia-induced changes to the circadian clock and circadian rhythms are complex and will not be fully explained by either the NMDA or GABA pathways.

An interesting difference between natural sleep and anaesthesia is that upon awakening from general anaesthesia patients have no perception that time has passed whilst unconscious. It is tempting to speculate that anaesthesia "pauses" the circadian clock, dissociating the patient from endogenous time perception cues. Indeed there is evidence from animal studies that this is the case (89). Future studies which explore the effect of anaesthesia on other aspects of behaviour and physiology such as cognitive function, may uncover novel roles for the circadian clock in the control of these processes. It is worth noting note that anaesthesia-induced activation of GSK3 β has been implicated as a major cause of neuronal apoptosis and post-operative cognitive impairment (90). Here we speculate that GSK3 β activation may be involved in the anaesthesia-induced disruption of the molecular circadian clock. It is therefore possible that post-operative cognitive impairment is at least partially a result of anaesthesia-induced disruption of the clock.

Practice points

General anaesthesia almost certainly contributes to circadian rhythm disruption in patients, resulting in disturbed sleep and potentially impeding patient recovery. Understanding the mechanisms of these effects, both anaesthesia's effect on the clock and the clock's effect on anaesthesia is important. Not all anaesthetics have the same effects on the clock. General anaesthesia is more likely to causes phase delays in patients having their operations during the day than at night. Shorter operations with shorter anaesthetic time are less likely to affect the clock. Mitigating anaesthesia-induced circadian clock disruption may provide a means of improving patient outcome.

Research agenda

Overall the priorities for research are both to increase our understanding of specific pharmaco-physiological mechanisms, and also to tease out the clinical impact of practical interventions. This would involve the study of:

- The interaction between anaesthetics and light entrainment of the circadian clock.
- The mechanism by which anaesthesia induces molecular changes in the SCN clock including the role of NMDA and GABA and the possible role of GSK3β signalling.
- How anaesthesia-induced changes in the SCN clock translate into downstream effects on physiological and behavioural rhythms at the whole organism level using standardised protocols.
- The persistence of the effect of anaesthesia on circadian rhythms.
- A comparison of the effect of anaesthesia between nocturnal and diurnal animals with an emphasis on diurnal animals to provide a better model for humans.
- the effect of anaesthesia on physiological circadian rhythms as this may differ from the effect on behavioural rhythms
- Better description of actual circadian rhythms and their disruption in hospitalised surgical patients, and the expected variability associated with different illnesses and operations.
- A comparison of the effects of general anaesthesia and regional anaesthesia (e.g. for hip surgery)
- The effects of both general and regional anaesthesia on peripheral circadian clocks and physiological rhythms in peripheral tissues.
- The link between preservation of circadian rhythm and clinically important immunological outcomes of post-operative infections and cancer spread.

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TABLE 1 Summary of results from all published studies investigating the effect of anaesthesia on rest/activity rhythms in animals.

Anaesthetic	Organism		Duration of anaesthesia	Effect on phase of rest/activity cycle: Anaesthesia during active period	Effect on phase of rest/activity cycle: Anaesthesia during inactive period	Effect on overall level of activity: Anaesthesia during active period	Effect on overall level of activity: Anaesthesia during inactive period	Ref
Isoflurane 2%	Bees	Free-running	6hr	Delay	NONE	-	-	(7)
Isoflurane 1.4%	Rats	Entrained	4hr	Advance	NONE	-	-	(12)
Isoflurane 1%	Mice	Entrained	6h	-	-	Increase (during subsequent rest period)	-	(17)
Sevoflurane 2.5%	Mice	Free-running*	4hr	Delay	Delay	-	-	(11)
Sevoflurane 4%	Rats	Free-running	8hr	-	NONE	-	Decrease	(8)
Sevoflurane 2.5%	Mice	Free-running#	4hr	-	Delay	-	Decrease	(15)
Pentobarbital i.p. 50mg/kg	Rats	Entrained	~5hr	NONE	NONE	Decrease	NONE	(13)
Ether (20ml)	Rats	Entrained	~50mins	-	NONE	-	NONE	(18)

Ketamine i.p. 100mg/kg	Rats	**Entrained**	~50mins	-	**NONE**	-	**NONE**	(18)
Ketamine i.p. 150mg/kg	Rats	**Entrained**	~4-4.5h	**Advance**	**Delay**	**Decrease**	**NONE**	(13)
Propofol i.p. 120mg/kg	Rats	**Entrained**	25-30mins	**Advance**	**Advance**	-	-	(19)
Propofol i.p. 60mg/kg	Rats	**Entrained**	30mins	**Advance**	**Advance**	-	-	(20)

* Entrained to a 14h light/10h dark cycle. Free-running in constant darkness from day of anaesthesia onwards

Entrained to a 14h light/10h dark cycle. Free-running in constant darkness from four days prior to anaesthesia onwards.

- Not reported