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Circadian rhythms and memory formation

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Abstract

There has been considerable progress in elucidating the molecular mechanisms that contribute to memory formation and the generation of circadian rhythms. However, it is not well understood how these two processes interact to generate long-term memory. Recent studies in both vertebrate and invertebrate models have shown time-of-day effects on neurophysiology and memory formation, and have revealed a possible role for cycling molecules in memory persistence. Together, these studies suggest that common mechanisms underlie circadian rhythmicity and long-term memory formation.

Circadian rhythms are basic biological phenomena that exist throughout phylogeny. They are influenced by zeitgebers and regulate various physiological events that include the cell cycle, body temperature, metabolism, feeding and, perhaps most notably, the sleep–wake cycle. Far less well understood is the relationship between circadian rhythm biology and memory formation. The impact of time-of-day effects and of circadian rhythms on cognitive performance in humans^{1–3} and on memory in animals^{4–7} have been studied for decades, and there has been a renewed interest in this topic in light of an increased understanding of the genetic, molecular and systems-level events that underlie these complex processes⁸. Recent discoveries have shown a high level of integration between cellular signalling cascades (such as the cyclic AMP–mitogen-activated protein kinase (MAPK)–cAMP-responsive element-binding protein (CREB) pathway) that regulate circadian rhythms and memory processing. Disruption of circadian rhythms or specific signalling cascades that undergo time-of-day-dependent cycling, by behavioural, environmental, genetic or pharmacological means, has negative consequences on memory and cognitive performance in various tasks and in several species. Given that modern society is becoming less dependent on the natural 24-hour light–dark cycle, an increased understanding of the functional relationship between circadian rhythms and cognitive function has broad implications for public health⁹.

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Here, we summarize studies that have shown a time-of-day effect on memory formation and compare the emerging common themes in various invertebrate and vertebrate species. We first describe the molecular pathways and time-of-day-dependent neuronal activity patterns that are conserved in circadian pacemaker cells in flies and rodents. Next, we present work that shows circadian alterations on neurophysiological processes that involve synaptic plasticity (such as long-term potentiation) and on memory formation in nocturnal (night-active), diurnal (day-active), and crepuscular model systems. On the basis of the cycling pattern of molecular cascades that are involved in memory formation, we address whether the cyclical reactivation of these cascades over the 24-hour day is essentially independent from inputs of the core time-keeping cells that are known to contribute to locomotor rhythm output. This Review expands on previously understood circadian effects on memory at the behavioural and physiological level, by focusing on recent data that show a possible involvement of circadian cycling of specific molecular pathways in long-term memory formation. Further background information has been published elsewhere on circadian rhythms^{9,10} and memory formation^{11,12}.

Are clock genes memory genes?

The initial characterization of the molecular players involved in the generation of circadian rhythms was carried out in the *Drosophila melanogaster* model. Over three decades ago, work on fruitflies showed that the periodic timing of the eclosion rhythm was dependent on the strain of fly. This suggested a genetic basis for the circadian regulation of this process, prompting a forward mutagenesis screen that identified the first clock gene, *period* (*per*)¹³. This gene was eventually cloned independently by separate laboratories^{14,15}. Levels of *per* mRNA and protein were shown to cycle in a circadian manner in flies and mammals and to be a part of a phylogenetically conserved transcriptional auto-regulatory feedback loop (FIG. 1) that is necessary for the synchronized expression of the circadian rhythm of locomotor activity^{16,17}. In *D. melanogaster*, mutations in *per* result in differences in the length of the eclosion rhythm, and include long (*per^L*), short (*per^S*), and arrhythmic (*per⁰*) phenotypes. Interestingly, these mutations cause correlative changes in the periodicity of the circadian locomotor activity rhythm in adult flies. Under constant dark (DD) conditions, *per^S* flies have a shortened circadian rhythm, *per^L* have a lengthened circadian rhythm, and *per⁰* are arrhythmic. This evidence suggests that the single clock gene *per* has pleiotropic effects on the timing of two separate processes at different developmental stages. Do clock genes have a role in the time-of-day effects on memory formation? Curiously, in contrast to wild-type flies, in mutant *per⁰* flies and *tim⁰¹* flies — which have a mutation in the gene encoding Timeless (TIM), the binding partner of PER proteins — there is no time-of-day effect on short-term olfactory avoidance memory under DD conditions¹⁸. In addition, as measured in a courtship conditioning assay, *per⁰* flies are defective in long-term memory (LTM) formation — a phenotype that can be rescued with a wild-type copy of the *per* gene in the *per⁰* background¹⁹. Overexpression of *per* in this paradigm has even been shown to enhance LTM¹⁹ despite these flies retaining rhythmic locomotor and mating activities under free-running conditions. This suggests that *per* regulates memory independently of its role in eclosion or in the generation of circadian rhythms.

Previous work has shown that there is a role for another transcription factor, CREB, in the core circadian clock of flies²⁰ and mammals²¹. In addition, a functional cAMP-responsive element (CRE) site in the promoter of mouse *per* genes that binds CREB has been described²², suggesting a link between CREB activity and PER activity in circadian rhythm generation (FIG. 1). A functional relationship between CREB activity and *per* expression was also shown in *D. melanogaster*²⁰. Flies that carry a luciferase reporter downstream of the *per* gene promoter (*per*-luc) have a disrupted and reduced amplitude of circadian transcriptional activity in a CREB-mutant background, indicating a functional link between CREB activity and circadian gene expression in *D. melanogaster*. In addition, *per* expression affects the cycling of CRE-mediated activity. Flies that carry a luciferase reporter downstream of three CRE sites (CRE-luc) normally show a circadian rhythm of luminescence under conditions of 12-hour light followed by 12-hour dark (LD) as well as under DD conditions. This CRE-luc cycling is coordinately altered in *per* mutants: in *per^L* flies, the CRE-luc cycling pattern is lengthened, whereas it is shortened in *per^S* flies, compared with wild-type controls, and in *per⁰* flies the CRE-luc activity pattern is arrhythmic across the day²⁰. A mutation in the *Creb2* gene of *D. melanogaster* also produces a shortened circadian cycle of locomotor activity, suggesting that CREB regulates normal circadian behaviour in flies²⁰. These data support a reciprocal relationship between CREB- and PER-mediated transcriptional regulation, with functional relationships in the generation of circadian rhythms. The precise relationship between CREB- and PER-mediated transcriptional activity (for example, through the cAMP-MAPK-CREB cascade) in the time-of-day-dependent regulation of memory is still unclear.

Anatomical relay of circadian centres

It is well established that certain molecules with cycling activity patterns influence circadian rhythms, but what is known about how neural networks generate the ultimate behavioural output? Considerable progress has been made in elucidating the cellular components and neuronal pathways that are responsible for the generation of circadian rhythms, and some similarities have been found across many species. Across phylogeny, clock-containing circadian pacemaker cells in the central nervous system receive photic input and can drive changes in locomotor rhythms over the course of the day. For example, in mammals, photic activation of non-image-forming retinal ganglion cells, which contain the photo-responsive pigment melanopsin, send light information to the central pacemaker of circadian rhythms — the suprachiasmatic nucleus (SCN)²³ — via the retinohypothalamic tract (RHT)²⁴. The core of the SCN receives photic input from the RHT and relays it to the subparaventricular zone (sPVz), which in turn relays the information to other hypothalamic structures (FIG. 2). These hypothalamic structures are known to regulate many physiological processes, including thermoregulation, hormone secretion, feeding behaviour and arousal- sleep states. The SCN is therefore thought to regulate the circadian timing of these processes. Pathways connecting the SCN to limbic structures that are involved in memory processing, such as the hippocampus and amygdala, have been shown^{25,26}. Other indirect connections — such as through hypocretin-expressing cells in the lateral hypothalamus²⁷ or through superior cervical ganglion-stimulated melatonin release from the pineal gland²⁸ — could relay SCN-derived circadian input to the hippocampus (FIG. 2). Whether these connections are

responsible for the time-of-day-dependent expression of memory and/or synaptic plasticity is not known. In *D. melanogaster*, photic input entrains a circadian rhythm in circadian pacemaker cells (of which there are ~150) through at least three pathways²⁹: the eyes³⁰, the Hofbauer-Buchner eyelets^{31–33} and/or the blue-light photo pigment cryptochrome (CRY)^{30,34}. Photoreceptive cells in the optic lobe are thought to project to the lateral neuronal cells via the posterior optic tract³⁵ (POT) (FIG. 2). This has been supported by recent findings that describe functional connectivity between the contralateral optic lobe and the large ventral lateral neurons (LNV) via the POT³⁶. Additionally, the Hofbauer-Buchner eyelets send projections to the accessory medulla³⁷, where they synapse with the small LNV pacemaker clock neurons^{38,39}. Anatomical connections among pacemaker cells that receive and relay this photic input to other brain regions — such as the mushroom bodies, a pair of neuropil structures in the insect brain known to regulate sleep^{40,41} and memory formation^{42,43} — in *D. melanogaster* are not well characterized. Although the small LNV cells have been shown to project to terminals near the mushroom bodies⁴⁴, a direct functional connection between pacemaker cells and cells that are responsible for memory formation is still lacking. The genetically tractable model of *D. melanogaster* will be valuable in determining the neural circuitry that is responsible for the integration of these two complex processes.

Time-of-day effects on neurophysiology

It is unclear whether a time-of-day-dependent relay exists between clock pacemaker neurons and memoryforming cells, such as between the SCN and the hippocampus of mammals or between the lateral neurons and the mushroom bodies of flies. However, cyclical changes over 24-hour periods have been observed in baseline physiological properties of central pacemaker cells in both mammals and flies. In nocturnal rodents, SCN neurons show circadian changes in spontaneous firing rate (SFR) and resting membrane potential (RMP), with an elevated SFR and more depolarized RMP in the light phase than the dark phase^{45–50} (FIG. 3). A similar effect was observed in the large LNV pacemaker cells in crepuscular *D. melanogaster*^{36,51,52}. Taken together, these data further support a phylogenetically conserved mechanism of circadian neurophysiology in pacemaker cells (FIG. 3). This conserved mechanism may influence time-of-day-dependent expression of physiological events downstream of central pacemaker cells, such as stimulation of brain regions that are involved in learning and memory.

Melatonin is a signalling molecule that is widely expressed throughout phylogeny and is secreted in a time-of-day-dependent manner⁵³. Recently, melatonin synthesis has been suggested to interact with core circadian mechanisms⁵⁴. The temporal release of melatonin is regulated over the 24-hour day in humans⁵⁵ and many other species, including zebrafish⁵⁶ (*Danio rerio*), sea slugs⁵⁷ (*Aplysia californica*), mice⁵⁸ (*Mus musculus*) and flies⁵⁹ (*D. melanogaster*). Interestingly, melatonin affects the firing rate of the mammalian SCN^{60–62}, suggesting that the time-of-day-dependent regulation of hormonal secretion may alter the firing patterns of circadian pacemaker cells. Melatonin also affects the firing rate of hippocampal CA1 neurons⁶³. Therefore, circadian hormonal modulation of neuronal firing could be a general mechanism throughout the brain. It would be interesting to know whether there are circadian fluctuations in the baseline SFRs and RMPs in hippocampal or

mushroom body neurons, and whether these oscillations occur in phase with those of the SCN and large LNvs, respectively. If these circadian firing patterns occur, an important next step will be to address whether they result from functional connectivity between pacemaker cells and memory-encoding regions, or whether they are caused by autonomous cycling molecules or circulating hormones such as melatonin. Further studies are needed to examine the functional connectivity between these brain regions that are involved in circadian rhythms and memory, and to compare baseline neurophysiological properties.

Time-of-day effects on synaptic plasticity

Given that circadian changes in molecular and neurophysiological properties of pacemaker cells are observed throughout the animal kingdom, an obvious question is whether neural correlates of plasticity-related neurophysiology are also regulated by circadian rhythms. In the hippocampus, long-term potentiation (LTP) — a form of synaptic plasticity (BOX 1) that is thought to underlie learning and memory⁶⁴ — has been shown to change depending on the time of day in various nocturnal rodents^{65–70} (FIG. 4). These circadian effects on LTP can be considered a naturally occurring form of metaplasticity⁷¹, in that the synaptic efficacy for a given amount of stimulation varies based on the time of day. Circadian changes in plasticity may serve as a useful model with which to study the neurophysiological and molecular mechanisms of metaplasticity. It would also be interesting to compare plasticity-related mechanisms in the SCN with those in the hippocampus. Molecular pathways (FIG. 1) that are involved in establishing plastic changes in circadian neurophysiology may be more fundamental than previously appreciated and could be shared with other known plasticity-related brain regions, such as those involved in memory formation. Lastly, knowledge of how baseline conditions can be modulated by the time of day is crucial for understanding the effect on synaptic plasticity of other experimental manipulations.

Synaptic plasticity in the suprachiasmatic nucleus

Time-of-day-dependent changes in synaptic plasticity have been observed in the SCN. Stimulation of the optic nerve can elicit potentiated responses in the SCN that last for hours, analogous to those observed following Schaffer collateral stimulation of the CA1 region of the hippocampus (BOX 1). In the SCN of rats, following stimulation of the optic nerve and with changes recorded in field potentials at six time points over the course of the day (starting 1 hour after normal lights-on), a potentiation of synaptic strength was observed during the day phase of the circadian cycle⁶⁹ (FIG. 4). However, this study was unable to detect the previously reported time-of-day-dependent changes in LTP in the hippocampus⁶⁷, which raises questions about how time-of-day effects on LTP may be regulated.

Hippocampal long-term potentiation

Hippocampus-dependent tasks, such as inhibitory avoidance conditioning, are thought to elicit LTP-like responses following training⁷², suggesting a functional relationship between synaptic plasticity mechanisms and memory formation. In 1977, it was shown that synaptic excitability of the rat hippocampal dentate gyrus exhibited a circadian rhythm⁶⁵. Previously, a meta-analysis examined over 170 studies for a circadian component of hippocampal LTP in rats, and found that the incidence and the magnitude of LTP were dependent on the time

of day⁶⁷. Interestingly, LTP was elevated during the dark phase in the dentate gyrus but was elevated during the light phase in area CA1 (FIG. 4). A clear influence of the time of day on LTP in the hippocampal CA1 region was also observed in the Syrian hamster, with increased LTP in animals that were killed during the light phase than during the dark phase⁷³. However, unlike the rat studies analysed previously⁶⁷, this study used tissue that was harvested during the opposite time of day from when the electrophysiology was completed. 'Daytime' hippocampal slices were prepared between zeitgeber time (ZT) phase 4.5–5.5, but LTP was not evaluated until the night-time (between ZT13.5–19.5). Conversely, 'night-time' hippocampal slices were prepared between ZT18.5–19.5, but LTP was not evaluated until ZT4. LTP in the CA1 was greater in hamster tissue that was harvested during the light period and then tested later during the dark period than in hamster tissue that was harvested and tested in the reverse conditions⁷³ (FIG. 4). This prompted examination of whether the time-of-day effects on LTP are dependent on the time of tissue harvest or the time of testing⁷⁰. In hippocampal CA1 tissue harvested from nocturnal C3H and C57Bl/6J strains of mice during the light period, LTP was greater in tissue that was examined during the dark period than during the light period⁷⁰ (FIG. 4). These data are in contrast to the meta-analysis of nocturnal rat LTP⁶⁷, but are consistent with data on nocturnal hamsters⁷³, and together suggest that the time-of-day effects on LTP are dependent on the time of testing, and not the time of harvest. Furthermore, these results support the hypothesis that an independent circadian pacemaker controls the time-of-day-dependent changes in hippocampal plasticity and that arousal state or sleep per se are not necessary for circadian changes in LTP.

Hormonal regulation of long-term potentiation

Hormones have been shown to modulate LTP in the SCN and hippocampus. For example, melatonin has been shown to block LTP in both regions^{74–77}. C57Bl/6J mice, which lack melatonin, undergo less dramatic time-of-day-dependent changes in hippocampal LTP than C3H mice, which express melatonin⁷⁰. Melatonin also inhibits neocortex-dependent LTP and memory formation in rats⁷⁸, suggesting a broad action of melatonin on synaptic plasticity in other brain regions. These data are interesting given the influence of melatonin on memory formation⁷⁹ and the timing of melatonin peaks in the circadian rhythm of memory formation in various species (FIG. 5). In addition, studies in adrena-lectomized (ADX) rats have implicated hormonal modulation in the time-of-day effect on hippocampal LTP⁶⁸. In ADX rats, the peak of LTP in the dentate gyrus shifted from the night-time to the daytime, suggesting that hippocampal LTP is regulated by other circulating hormones, such as those found in the adrenal gland. As the circadian regulation of adrenal corticosterone is under the influence of the SCN⁸⁰, the altered cycling of hippocampal LTP that is observed in ADX rats may result from a disruption in signalling from the sCN to the hippocampus via the adrenal gland. Given that the time of testing, rather than the time of harvest, seems to affect hippocampal LTP, it is plausible that cycling hormones affect the 'clock-time' of hippocampal cells before tissue harvest. These data suggest that circulating hormones could operate as a zeitgeber, setting the clock in hippocampal cells such that they retain the rhythm during testing that was previously entrained by hormone signalling. Whether there is a relationship between rhythms of LTP and rhythms of memory formation remains to be determined.

Circadian effects on memory formation

Time-of-day and circadian effects on cognitive performance and memory formation have been observed in various behavioural paradigms^{81–84}. The influence of sleep and behavioural state on memory consolidation are reviewed elsewhere^{85–87}.

Interestingly, circadian rhythms of locomotor activity are not an accurate predictor of the timing of optimal performance in memory tasks over the course of the day. The circadian rhythms in memory formation in nocturnal, diurnal and crepuscular species do not show a clear correlation with their specific rhythm of locomotor activity. For example, peak performance in memory tasks occurs during the ‘active phase’ of the diurnal zebrafish⁷⁹ (*D. rerio*) and sea slug^{18,88} (*A. californica*), but during the ‘inactive phase’ of the nocturnal house mouse⁸⁹ (*M. musculus*) and the crepuscular fruitfly⁹⁰ (*D. melanogaster*) (FIG. 5). This suggests that activity levels are not responsible for the changes in memory formation that occur over the course of 24 hours, as there is a clear dissociation of the rest-activity rhythm and cognitive performance in the various chronobiological models. This indicates that factors other than the behavioural state are involved, supporting the theory that specific cellular or molecular events that cycle over the 24-hour day are responsible for the enhancement of memory at specific times. Processes that could contribute to this memory enhancement include gene transcription and translation, epigenetic mechanisms, neurotransmitter release, synaptic excitability, neuronal activity and hormone secretion. In addition, each of these processes may have different effects on various phases (acquisition, consolidation and retrieval) of memory.

Does melatonin influence memory? A better predictor of the circadian variation of peak performance in memory tasks may be cycling molecules with a periodicity that follows the rhythm of performance — rather than the rhythm of locomotor activity — across species. One molecular correlate that seems to be a good candidate is melatonin. Interestingly, levels of melatonin are inversely related to cognitive function: peak melatonin release occurs during the lower periods of performance over the course of the day in humans^{8,91} and other species (FIG. 5). This suggests that levels of melatonin are good predictors of the nadir (lowest) period in the time-of-day cycling of memory.

Recently, the role of melatonin in the regulation of memory was investigated using an active-avoidance conditioning paradigm in zebrafish⁷⁹. Fish were trained in a tank to make an ‘unsafe’ association in a dark compartment, in which they received electric shocks, and a ‘safe’ association within a lit compartment, in which there were no electric shocks. A clear time-of-day effect was observed in acquisition (learning) and memory formation, and both were improved during the daytime (active) period. These effects were maintained under DD — a condition that is necessary to evaluate whether an endogenous circadian system controls the time-of-day effect. Treatment of fish with melatonin before training had no effect on acquisition but significantly reduced LTM formation. Fish that were treated with melatonin, either following training or just before testing, did not show differences in LTM or retrieval, respectively. This finding supports a role for melatonin in the earlier stages of LTM formation. Furthermore, this phenotype was rescued by treating the fish with melatonin receptor antagonists, either simultaneously with melatonin during the daytime or alone

during the night-time phase (when endogenous melatonin is high and LTM is low). These results suggest that melatonin signaling cascades influence the time-of-day effects on memory formation. However, the melatonin theory is controversial as C57B1/6J mice exhibit time-of-day-dependent changes in memory formation⁸⁹ despite the absence of melatonin^{58,92,93}, and so further studies are warranted.

Time of acquisition versus time of recall

Differences in time-of-day effects on memory acquisition versus recall have been observed on various tasks in various models^{79,88,89,94–96}. In *A. californica*, the circadian rhythm of long-term sensitization (LTS) was examined⁸⁸. LTS training consists of a series of shocks delivered to the side of the sea slug, which elicits a siphon withdrawal response, followed by a post-training electrical shock to the tail, which elicits a ‘sensitized’ siphon withdrawal. Memory is expressed as the ratio between pre-training and post-training durations of the siphon withdrawal behaviour. A time-of-day effect on LTS memory was observed, with peak responses during the ‘active period’ in both LD and DD conditions (FIG. 5). Time-of-day-dependent regulation of the baseline siphon withdrawal response or the withdrawal duration was not observed in either LD or DD conditions, suggesting that the memory results from an endogenous circadian mechanism. This time-of-day effect on LTS depended on the time of acquisition, rather than on the time of recall. The LTS response was greater in animals that were trained at circadian time 9 (CT9; when LTS is enhanced) and tested at CT21 (when LTS is suppressed) than in animals that were trained and tested at CT21. Animals that were trained at CT21 and tested at CT3 (when LTS is normally enhanced) did not show an elevated LTS response, suggesting that the time of training (learning) and not the time of testing (recall) is responsible for the circadian rhythm of LTS in *A. californica*. This is in contrast to the result in mice⁸⁹, which suggested that optimal recall times are under circadian control.

The mouse inbred strains C3H and C57B1/6J have significant time-of-day effects on memory formation in a fear conditioning paradigm⁸⁹. They display optimal memory recall during the ‘inactive phase’ (the light period) for contextual and cued fear conditioning. These mice also show a time-of-day effect on memory acquisition, with an elevation during the light period, at ZT3, compared with the dark period, at ZT15. A possible relationship between the effect of time of acquisition and the effect of the time of recall was also examined. Mice were trained during the day period, at ZT3, under LD conditions. They showed circadian cycling of memory that was maintained for at least 3 days, with a period of ~24 hours and a peak enhancement reoccurring at the same time as training. Interestingly, in DD conditions with training at CT3, mice displayed the same ~24-hour periodicity of peak memory, reoccurring at CT3 for 3 consecutive days⁸⁹. This suggests that the peak time of acquisition may coincide with the peak time of recall. To test this, mice were trained during the time of poorer acquisition (ZT15), and examined for effects on recall. The circadian cycling of the peak time of recall (ZT3) was preserved to the third day of testing. To determine whether this effect was truly circadian, animals were trained in DD conditions at CT15 and, surprisingly, still showed memory enhancement at CT3 on subsequent days of testing. This suggests that the peak time of memory recall is under time-of-day-dependent

control, that it is independent of the time of training and is regulated by an endogenous circadian system.

However, a time-of-day effect on recall not was observed in a subsequent study using fear conditioning in C57Bl/6J mice⁹⁶. Specifically, there was no elevation in recall at ZT4 when animals were trained at ZT16 (REF. 96). However, this study had lower temporal resolution than the study described above⁸⁹, which might account for the apparent conflict of results. It is also possible that variation in the strength of the training protocol could skew results, obscuring the circadian effects on memory processes. In addition, when animals are tested repeatedly⁸⁹, testing would include combined effects of recall, reconsolidation and extinction, making it difficult to discern the circadian influence on solely the recall stage of memory. Further studies examining the role of several memory stages, such as acquisition versus recall, are required to determine the relative contributions of these factors to the time-of-day effects on memory formation.

Molecular oscillators and memory

The cAMP-MAPK-CREB pathway

The cAMP signalling cascade has a central role in memory formation⁹⁷. For example, in *D. melanogaster*, overexpression of a repressor isoform of CREB (CREB2B) selectively abolishes LTM formation without affecting short-term memory⁹⁸. By contrast, overexpression of an activator isoform of CREB (CREB2A) enhances LTM⁹⁹, and this is dependent on the time of day¹⁰⁰. When stimulated near the light-dark transition (before training), conditional expression of CREB2A enhances the formation of 4-day-long memory. This effect does not occur when CREB2A is stimulated in the middle of the daytime (before training), suggesting a time-sensitive window when the encoding of CREB-mediated enhancement can occur. Additionally, it was shown that time-of-day-dependent cycling of various components of the cAMP cascade is necessary for LTM formation in mice⁹⁶. Circadian cycling of cAMP and MAPK phosphorylation paralleled time-of-day-dependent oscillations in RAS activity and the phosphorylation of MAPK kinase and CREB in the hippocampus. Disruption of the circadian rhythm of this cAMP-MAPK-CREB cascade in the hippocampus, by pharmacological approaches or by exposure of animals to constant light conditions, impaired memory formation.

In *A. californica*, although baseline activity of MAPK does not follow a circadian rhythm in the pleural ganglia, LTS training during different times of day produces different levels of activated MAPK¹⁰¹. For example, phosphorylation of MAPK increases following LTS training during the daytime as compared with the night-time, and this phosphorylation correlates with patterns of LTM enhancement. This poor performance during the night-time following LTS training can be rescued using compounds that activate MAPK activity or MAPK-dependent transcription. It therefore seems that, as in mice⁹⁶, the circadian clock is also able to modulate LTM formation in *A. californica* through the MAPK cascade. Taken together, these data strongly suggest that cAMP-MAPK-CREB is a phylogenetically conserved pathway for the time-of-day dependence of memory.

Epigenetic factors

Epigenetic factors provide an interesting link between the molecular mechanisms that underlie circadian rhythms and the formation of memory. In the mature nervous system these factors influence changes in synaptic plasticity and complex behaviour, such as drug addiction, memory and circadian rhythms¹⁰². Changes in the epigenetic state of specific cells may be a principal mechanism by which altered gene expression exerts a circadian influence on memory formation. Chromatin remodelling following stimulation occurs in hippocampal cells¹⁰³ and is induced by light in SCN cells¹⁰⁴. It is therefore possible that epigenetics is a common mechanism relaying the cyclical change in various memory-related processes. Rhythmic changes in chromatin states are altered in a circadian manner^{105–107}. Furthermore, the protein product of the circadian gene *CLOCK* itself has histone acetyltransferase activity¹⁰⁸ and has recently been shown — with its binding partner brain and muscle ARNT-like (BMAL) (FIG. 1) — to regulate LTM formation¹⁰⁹. Memory formation elicits histone modifications^{110,111}. It is possible that specific molecular pathways, such as the cAMP-MAPK-CREB cascade, may stimulate these epigenetic changes that ultimately drive LTM through circadian gene expression. Future study of the relationships between these molecular pathways and their influence on epigenetic mechanisms may provide new insight into the circadian regulation of memory persistence.

Central versus cell-autonomous oscillators

Which cells are responsible for driving the circadian regulation of memory? Is a central pacemaker required, or do cell-autonomous oscillators in the memory-encoding neurons themselves regulate the time-of-day effects on memory (FIG. 6)? Although SCN ablation inhibits cycling of *Per2* in the amygdala and hippocampus of hamsters¹¹², it does not prevent expression of the time-stamp memory in a conditioned place-avoidance task¹¹³. It is possible that the time stamp could be partially encoded by memory cells using the same molecular machinery that is involved in maintaining circadian rhythms within pacemaker cells. Furthermore, mouse knockouts of neuronal PAS domain protein 2 (NPAS2), which is not expressed in the SCN but is a binding partner of the essential clock protein BMAL, have LTM deficits in cued and contextual fear conditioning¹¹⁴. However time-place memory — a form of learning in which an association is formed between a specific location and the time of day — still requires the *Cry* genes in mice¹¹⁵ (FIG. 1). Together, these data suggest that the cycling of specific components of the core molecular oscillatory pathway is required, perhaps in the SCN or extra-SCN regions, for the expression of time-stamp memory. In addition, rhythms of passive avoidance conditioning in rats^{5,116} require an intact SCN¹¹⁷, supporting a role for central circadian pacemaker cells in the regulation of time-of-day effects on memory. Further work is therefore needed to determine how circadian transcriptional mechanisms in the core pacemaker (the SCN), versus the peripheral cells (extra-SCN), contribute to the persistence of LTM.

Conclusions and perspectives

The conservation of a time-of-day effect on memory in many species points towards a common molecular mechanism. This is likely to involve the cAMP-MAPK-CREB cascade,

which is implicated in the generation of memory and circadian rhythm processes and is phylogenetically conserved. Given the circadian influence on the regulation of memory-related molecules, neurophysiology, synaptic plasticity and behaviour, it is crucial that neuroscientists consider the effect of time-of-day-dependent variation on the experimental design, analysis and interpretation of future studies. Further study is needed to examine the signalling pathways involving molecules that have been studied for roles in memory and circadian rhythms, such as fragile X protein. This protein has been shown to regulate circadian rhythms and memory in flies^{118–121} and mice^{122–124}. In addition, levels of vasoactive intestinal peptide (VIP) cycle in the rodent SCN and contribute to the signalling of the cAMP-MAPK-CREB cascade^{23,125}. Interestingly, VIP knockout mice, which lack a circadian locomotor rhythm, retain time-of-day effects on memory, albeit to a lesser extent than wild-type mice¹²⁶. Further research is needed to determine whether these pleiotropic molecules exert circadian effects on memory through known core clock mechanisms or through secondary effects outside of the known circadian pathways.

What is the influence of core clock-controlled pacemaker cells on the neuronal networks that are responsible for memory formation? As circadian clocks are autonomous in many tissues¹²⁷, it is possible that cell-autonomous clocks in different regions of the brain independently control the circadian timing of neurophysiology and memory-related processes. For example, in *A. californica*, time-of-day effects on LTS persist in the absence of an ocular circadian oscillator¹²⁸, and in *D. melanogaster*, circadian rhythms in the olfactory system are autonomous from lateral neuron pacemaker cells¹²⁹. Similarly, in rodents, ablation of SCN pacemaker cells does not affect circadian oscillations in gene expression in the olfactory bulb^{130,131} and daily oscillations of gene expression in brain regions outside of the SCN are anti-phase to those in the SCN^{130,132}. Interestingly, *Per2* expression oscillates in isolated hippocampal tissue¹³³, suggesting that circadian gene expression in these extra-SCN regions may be autonomous. Further studies that analyse the contribution of endogenous oscillators in memory-forming brain regions (versus central pacemaker cells) to the time-of-day effects on memory are vital for our understanding of normal brain physiology. For example, does the learning event itself serve as a zeitgeber in memory-encoding regions, setting a memory ‘clock’ of its own such that the memory is better retrieved at certain times of day, as time-stamp conditioning suggests? Are the epigenetic mechanisms that are involved downstream of the light zeitgeber in the clock pacemaker cells (for example, the SCN) analogous to the epigenetic mechanisms that produce an engram in memory centres such as the hippocampus? Studies of functional connectivity between clock cells and learning and memory centres over circadian time following training, consolidation and retrieval in various organisms will improve our understanding of these systems-level relationships and verify the appropriate model (FIG. 6).

Studies that specifically examine how the circadian cycling of molecules may affect the persistence of memory are also needed. Recently, disruption of circadian locomotor behaviour by constant light conditions has been shown to disrupt the circadian rhythm of phosphorylation of MAPK in the hippocampus and to decrease the persistence of LTM in fear-conditioned mice⁹⁶. In rats, constant light conditions induce retrograde amnesia following passive avoidance conditioning⁷ and impair hippocampus-dependent spatial memory performance in the Morris water maze¹³⁴. However, chronic light exposure was

unable to disrupt an aversive-conditioning memory in mice¹³⁵. Furthermore, disruption of circadian rhythms by phase-shifting in mammals disrupts various forms of memory^{6,7,136–138}, although it was not possible to impair memory in fear conditioning using this particular method¹³⁷. There are data suggesting that circadian rhythms might have a role in other forms of memory, such as social recognition, although this is controversial^{84,139}. As the hypothalamic-pituitary-adrenal axis is regulated by the circadian system¹⁴⁰ and influences synaptic plasticity and memory^{141,142}, the role of stress and stress-related hormones in the time-of-day effects on memory warrant further investigation. Future studies should also determine the involvement of circadian mechanisms in ageing-associated cognitive decline^{143,144}. Studies to examine the influence of circadian clock machinery on memory and other complex behaviours will help to discern which behaviours are under time-of-day control, and whether the circadian cycling of molecules themselves function to facilitate memory persistence through ‘reverberating’ circuits or cascades¹⁴⁵. This type of research is particularly relevant to public health as society is increasingly operating outside of the normal daily light-dark cycle, and disrupted circadian rhythms exacerbate poor cognitive function. Future work on the mechanisms that underlie time-of-day effects on cognitive performance and memory processes will undoubtedly guide treatments for various neurological and sleep-related disorders.

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Box 1 |**Physiological analysis of long-term potentiation**

Two examples of the methods used to study the physiological manifestations of long-term potentiation (LTP) are shown in the figure. Rodent brain slices are obtained at the level of the suprachiasmatic nucleus (SCN; **a**), or the hippocampus (**b**). Following stimulation (STIM) of the optic tract or the Schaffer collateral pathway, recordings are made from electrodes (REC) that are implanted in cells of the SCN or area CA1 of the hippocampus, respectively. Example graphs depicting representative traces of recordings following stimulation show LTP in the form of a population spike or a field potential. Example traces (inset) are shown for the pre-stimulation baseline (shown by the orange line) and post-stimulation LTP (shown by the green line). LTP is expressed as a percentage increase of the slope or amplitude of electrical potential from the baseline over time.

Zeitgeber

A German word that means 'time-giver'. It refers to an exogenous cue, such as the light-dark cycle, that entrains a circadian rhythm.

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Circadian rhythm

The regular cycling of biological processes in an organism over a ~24-hour period that occurs regardless of the zeitgeber.

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Time-of-day effect

The effect of the specific point in time during the day–night cycle on the biological processes of an organism. The effect can be dependent or independent of a zeitgeber.

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Long-term potentiation

A persistent increase in synaptic strength following high-frequency stimulation of a synapse.

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Crepuscular

Describes an organism that is active during twilight or during day-to-night or night-to-day transitions.

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Eclosion rhythm

The timing of the emergence of the adult fly from its pupal case, which usually occurs at dawn.

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Clock gene

A gene that regulates aspects of circadian rhythms.

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Suprachiasmatic nucleus

A hypothalamic bilateral structure that is the central pacemaker of circadian rhythms in mammals.

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Melatonin

A catecholamine hormone derived from serotonin.

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Hofbauer-Buchner eyelets

Photoreceptor cells that are located between the retina and the lamina of the fly eye.

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Metaplasticity

Alterations in the ability of the synapse to change in strength.

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Figure 1 |. Phylogenetic conservation of the core molecular clock.

The molecular clock in flies and mammals is composed of transcriptional and translational feedback networks. In flies, CLOCK (CLK) and CYCLE (CYC) heterodimerize and activate transcription of the period (*per*) and timeless (*tim*) genes by binding to E-box elements in their promoters. The protein products PER and TIM heterodimerize and enter the nucleus following phosphorylation (P) by proteins such as doubletime (DBT) or casein kinase II (CKII), and repress the transcriptional activity of CLK-CYC. In mammals, the circadian clock comprises a similar feedback network, including CLOCK and the CYC homologue brain and muscle ARNT-like (BMAL), which activate the transcription of *per* and cryptochrome (*cry*) genes via E-box elements. PERs and CRYs heterodimerize in the cytoplasm following phosphorylation by proteins such as casein kinase Iε (CKIε), and enter the nucleus where they inhibit CLOCK-BMAL transcriptional activation. Mitogen-activated protein kinase (MAPK) phosphorylates BMAL¹⁴⁶, repressing BMAL-CLOCK activity. Putative mechanisms linking melatonin (MEL) rhythms and the circadian clock include repression of adenylate cyclase (AC) and protein kinase A (PKA), a pathway known to influence cAMP-responsive element (CRE)-binding protein (CREB) activation. A second mechanism is thought to activate the MAPK-CREB cascade^{147,148} through Ca²⁺ influx, leading to transcriptional activity through CRE elements in the *per* promoters²². An analogous putative pathway is shown for *Drosophila melanogaster*, in which MAPK phosphorylation represses CLK-CYC¹⁴⁹ or activates CREB, leading to *per* transcription.

Figure 2 | Anatomical circadian pathways in flies and mice.

a | In fruitflies (*Drosophila melanogaster*), various light-receiving cells are involved in functional neuroanatomical connections, such as those in the Hofbauer-Buchner (H-B) eyelets and ocelli (OC), or from the optic lobes (OL). These project to circadian pacemaker cells, the lateral neurons (LN), via the posterior optic tract (POT). LN subtypes include the large, small, and 5th small ventral LN (LNV), as well as the dorsal LN (LNd). Little is known about the functional connectivity between these pacemaker cells and other clock cells, such as the dorsal neurons (DN1, DN2 and DN3 subtypes) the lateral posterior neurons (LPN) or cells that are involved in sleep and memory formation, such as the pars intercerebralis (PI) and mushroom bodies (MB). DNs and LNs comprise the ~150 cells of the clock network in the fly brain. **b** | In the mouse (*Mus musculus*), the suprachiasmatic nucleus (SCN) receives photic input through the retinohypothalamic tract (RHT). The SCN projects to the dorsal medial hypothalamus (DMH) through the subparaventricular zone (sPVZ), which projects to various regions in the hypothalamus, including the ventrolateral preoptic area (VLPO), the lateral hypothalamus (LH) and the paraventricular nucleus (PVN). There are reciprocal connections between the VLPO and the tuberomammillary nucleus (TMN), which are thought to be partly responsible for the proper timing of sleep-wake rhythms. Functional connections between a circadian centre and a memory forming-centre, such as the hippocampus, are not well known. They may be partially gated through hypocretin- or orexin-expressing cells of the LH, or by melatonin secretion from the pineal gland following

signalling from the PVN to the superior cervical ganglion (SCG)¹⁵⁰. Note that C57BL/6J mice lack melatonin. OB, olfactory bulb.

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Figure 3 |. Time-of-day-dependent neurophysiology in pacemaker cells.

Fruitflies, which are crepuscular, have a higher spontaneous firing rate (SFR) and resting membrane potential (RMP) in their clock cells, such as the large ventral lateral neurons (LNV), near the dark-to-light transition and during the daytime, than in the light-to-dark transition and during the night-time^{36,52}. Similar to fly clock cells, neurons in the nocturnal rodent suprachiasmatic nucleus (SCN) have an elevation in SFR and RMP during the light period compared with the dark period, despite a difference in the locomotor activity rhythm of these two species^{49,50}. Top traces show a schematic representation of spike trace frequency over the day-night cycle. White bars represent 'lights on'; dark green bars represent 'lights off'.

Figure 4 | Time-of-day effects on synaptic plasticity.

Time-of-day-dependent changes in long-term potentiation (LTP) occur in various rodent species, including rats, hamsters and mice. **a** | Interestingly, in the nocturnal rat hippocampus, LTP is greater during the dark phase than in the light phase in the dentate gyrus (DG) but is greater during the light phase than in the dark phase in area CA1 (REF. 67). LTP is also time-of-day-dependent in the rat suprachiasmatic nucleus (SCN), with an elevation during the day phase compared to the night phase⁶⁹. **b** | In hippocampal area CA1 of the hamster, LTP is higher when tissue is isolated during the light phase and tested during the dark phase (light > dark) and lower when tissue is isolated during the dark phase and tested during the light phase (dark > light)⁷³. **c** | In area CA1 of the nocturnal mouse, LTP is lower during the light period, but enhancement of LTP (metaplasticity) occurs when tissue is harvested during the light period and tested during the dark period⁷⁰. Taken together, these data suggest that there are endogenous cellular oscillators that continue to function when dissociated from the intact brain, driving time-of-day-dependent changes in synaptic plasticity.

Figure 5 |. Melatonin and circadian rhythms of memory.

Low levels of melatonin correlate with high levels of memory performance and are seemingly independent of the activity state in vertebrate and invertebrate species. In the diurnal zebrafish (*Danio rerio*) and sea slug (*Aplysia californica*), there is a time-of-day-dependent enhancement of memory formation during the ‘active phase’ (a) of their circadian rhythm. By contrast, in the nocturnal mouse (*Mus musculus*) and the crepuscular fruitfly (*Drosophila melanogaster*), there is memory enhancement during the ‘inactive phase’ (b) of their circadian rhythm. Each species, regardless of activity state, has a corresponding anti-phase relationship between memory performance and melatonin levels. White bars represent ‘lights on’; dark green bars represent ‘lights off’. Figure is based on data from REFS 56,57,59,79,88–90,93.

Figure 6 |. Models for the circadian regulation of memory.

Cellular and molecular correlates of circadian cycling exist in various regions of the brain. Such regions include the mammalian suprachiasmatic nucleus (the central clock), as well as other cell populations, including those in the mammalian hippocampus (the memory centre). The degree to which the central clock is responsible for directing oscillations of the memory circuit, versus autonomous control by the memory cells themselves, remains to be determined. An integrated model seems most likely, whereby peripheral oscillators have some control that is coordinated with inputs (either directly or indirectly) from a central pacemaker. Orange circles represent central pacemaker-driven oscillators; blue circles represent autonomous oscillators. Solid arrows indicate direct control; dashed arrows indicate an influence on cellular oscillations.