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Regulation and function of extra-SCN circadian oscillators in the brain

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Abstract

Most organisms evolved endogenous, so called circadian clocks as internal timekeeping mechanisms allowing them to adapt to recurring changes in environmental demands brought about by 24-hour rhythms such as the light-dark cycle, temperature variations or changes in humidity. The mammalian circadian clock system is based on cellular oscillators found in all tissues of the body that are organized in a hierarchical fashion. A master pacemaker located in the suprachiasmatic nucleus (SCN) synchronizes peripheral tissue clocks and extra-SCN oscillators in the brain with each other and with external time. Different time cues (so called *Zeitgebers*) such as light, food intake, activity and hormonal signals reset the clock system through the SCN or by direct action at the tissue clock level. While most studies on non-SCN clocks so far have focused on peripheral tissues, several extra-SCN central oscillators were characterized in terms of circadian rhythm regulation and output. Some of them are directly innervated by the SCN pacemaker, while others receive indirect input from the SCN via other neural circuits or extra-brain structures. The specific physiological function of these non-SCN brain oscillators as well as their role in the regulation of the circadian clock network remains understudied. In this review we summarize our current knowledge about the regulation and function of extra-SCN circadian oscillators in different brain regions and devise experimental approaches enabling us to unravel the organization of the circadian clock network in the central nervous system.

KEYWORDS

brain, circadian clock, clock gene, CNS, entrainment

1 | INTRODUCTION

Most life on this planet is subject to dramatic changes in environmental conditions brought about by the rotation of the Earth around its axis. With the succession of day and night parameters such as illumination, temperature, humidity, but also the presence or absence of food resources, potential mating partners, or predators may alter markedly. To adapt to these pervasive, but highly predictable variations in external challenges most organisms have evolved internal timekeeping systems—so called *circadian clocks* (from the Latin '*circa diem*' meaning 'about a day')—to measure daytime and temporally coordinate physiology and behaviour.[1,2]

Circadian clocks affect a large spectrum of physiological outputs—from sleep-wake rhythms down to cell cycle regulation.[2,3] Consequently, the disruption of circadian rhythms—as seen for example in shift workers or during jetlag—can have consequences for health and well-being. Many common chronic diseases of modern societies such as type-2 diabetes,

major depression or cardiovascular disorders are promoted by chronodisruption, that is, the perturbation of internal clock function or of the alignment of these clocks with external time.[4] Besides shift work, other chronodisruptive factors are sleep curtailment, high-energy diets or mistimed eating patterns, and nocturnal light pollution.[5,6]

2 | THE MOLECULAR CLOCK

In complex multicellular organisms such as mammals, molecular clocks are found in most, if not all, tissues and cells. They are based on a set of core clock genes, namely *Brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein 1 (Bmal1, Arntl), Circadian locomotor output cycles kaput (Clock), Cryptochrome 1* and *2 (Cry1, Cry2)* and *Period 1, 2, 3 (Per1, Per2, Per3),* and their protein products that encode time via interlocked transcriptional-translational feedback loops (TTFLs).[7] In the core loop of the mammalian clock, the CLOCK:BMAL1 heterodimer binds to enhancer elements (*E-boxes*) regulating the transcription of *Per*, *Cry* and other clock-controlled genes (*CCG*s).[8] PER:CRY dimers accumulate in the nucleus over the course of the day to inhibit the activity of the CLOCK:BMAL1 heterodimer and thereby regulate their own transcription.[8] The degradation of the PER:CRY dimers during the second half of the day is controlled by the casein kinases CKIδ and CKIε and determines the period length of the circadian clock.[8] In a second TTFL, *retinoic acid-related orphan receptors* (*Rorα-γ*) and *reverse erythroblastoma* (*Rev-erbα/β, Nr1d1/2*) compete for binding to the *retinoic acid-related orphan receptor response element* (*RORE*) in the *Bmal1* promotor sequence and are thereby stabilizing the core TTFL since RORs activate whereas REV-ERBs inhibit *RORE*-mediated gene transcription.[9] In addition, another feedback loop acts via the regulation of destruction *(D)-boxes*, which were described in the promotor sequence of several clock genes.[10] Nuclear factor, interleukin 3 regulated (NFIL3, E4BP4) is repressing, whereas D-site albumin promotor binding protein (DBP) is activating *D-boxes*.[11] The diurnal activity of core clock genes as well as of *CCG*s is therefore controlled through binding of different protein regulators to *E-boxes*, *D-boxes* or *RORE*s. To adapt these molecular oscillations to external time, the circadian TTFL can be reset by light as a main stimulus, but also other stimuli such as polyunsaturated fatty acids (PUFAs), carbon monoxide (CO) and nitric oxide (NO) were described to have resetting functions (Figure 1).[12-15] Light stimulates the transcription of *Per* genes in the suprachiasmatic nucleus (SCN) resulting in entrainment of the circadian pacemaker to the day-night cycle.[9] *Per* induction is mediated by cAMP response element-binding protein (CREB) and mitogen-activated protein kinase (MAPK) interaction with *cAMP-response elements* (*CRE*s) in the promotor sequence of *Per1* and *Per2*.[9] PUFAs activate peroxisome proliferator-activated receptors (PPARs) which are then stimulating *Bmal1* transcription.[16] Additionally, PPARs have been reported to influence other clock components such as PER2 and REV-ERBα.[17] Both, NO and CO signalling pathways, lead to a production of cyclic guanosine monophosphate which then activates protein kinase G to induce phase shifts.[15]

Cellular clocks organize biological functions through the regulation of extensive, but highly tissue-specific transcriptional programs. It has been estimated that more than 40% of all protein-coding genes are subject to circadian regulation in at least one tissue of the body.[18] Chronodisruption may affect the clock system at different levels. Light pollution, for example, affects central clocks in the brain, but may additionally affect peripheral clocks indirectly while mistimed food intake is a dominant synchronizer (or *Zeitgeber*) for peripheral tissue clocks.[19,20]

3 | CLOCK HIERARCHY

The network structure of the mammalian circadian system is organized in a hierarchical fashion.[21] At its top sits the SCN, a bilateral structure of densely packed neurons at the frontal ventromedial tip of the hypothalamus straddling the optic chiasm. The SCN receives light input directly from intrinsically light-sensitive retinal ganglion cells (ipRGCs) that express the photopigment melanopsin (OPN4). ipRGCs are most sensitive in the blue range of the visible spectrum (λ_{max} = 480 nm) and capable of integrating light information over long periods of time.[22] Through the retino-hypothalamic tract ipRGCs reset clocks in the SCN, thus directly entraining them to the external light-dark cycle. The endogenous oscillation of SCN clocks determines behavioural and physiological rhythms in the whole animal.[23] Surgical lesioning of the SCN results in behavioural and molecular circadian arrhythmicity,[24] although short-period rhythms for some parameters may still be preserved.[25] From the SCN, neuronal and humoral connections reach subordinate cellular clocks in the brain and throughout the periphery, aligning them with each other and with external time.

Neuronal tracing studies could show that the SCN projects predominantly to hypothalamic regions but it also reaches some other brain regions such as, for example, the paraventricular nucleus of the thalamus (PVT) and the periaqueductal grey (Figure 2).[26-30] SCN projections to the subparaventricular zone were reported to be involved in generating rest-activity rhythms.[31] Projections from the SCN to the dorsomedial hypothalamus (DMH) and further to the locus coeruleus (LC) together with SCN projections

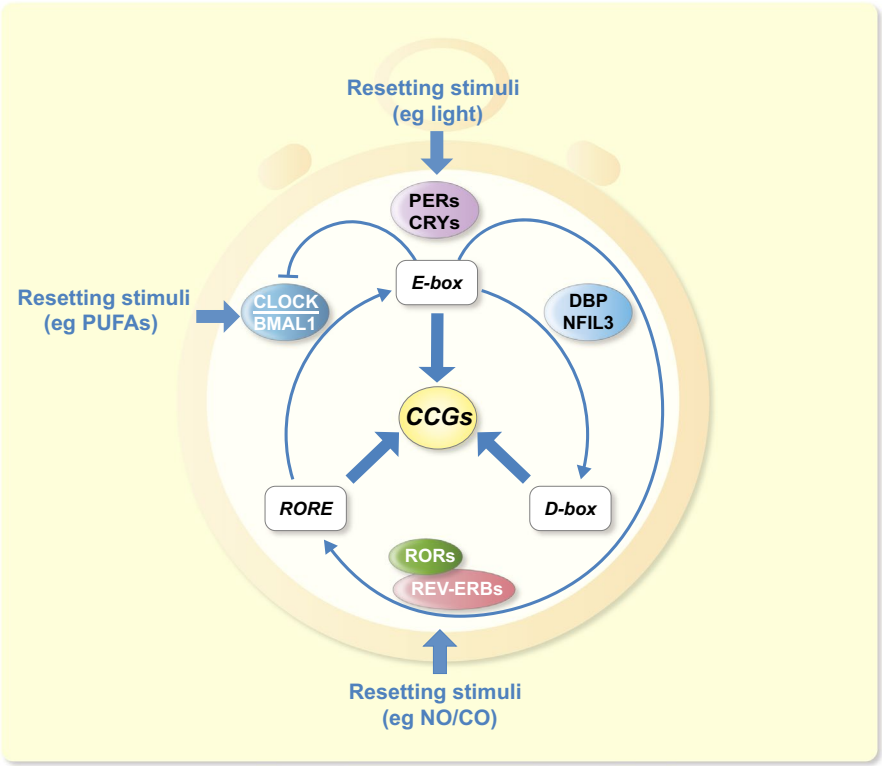

FIGURE 1 Regulation of the molecular clock. On the molecular level the transcription of the core clock genes and *clock-controlled genes* (*CCG*s) is influenced by different promotor elements such as *E-boxes*, *D-boxes* and *retinoic acid-related orphan receptor response element* (*RORE*). The CLOCK:BMAL1 heterodimer regulates the transcription of *Per*, *Cry*, *ROR*, *Rev-erb* and other (*CCG*s) by *E-box* binding. ROR and REV-ERB compete for *RORE* binding in the promotor of *CCG*s as well as in the *Bmal1* promotor and thereby either activate or inhibit transcription. A similar regulation takes place through DBP and NFIL3 competition for *D-box* binding in the promotor sequence of several *CCG*s as well as in the *Per* genes. While DBP is activating *D-boxes*, NFIL3 has an inhibitory effect. Several stimuli can reset the molecular clockwork such as polyunsaturated fatty acids (PUFAs) stimulating *Bmal1* via PPARs, light stimulating *Per* gene transcription, or nitric oxide (NO) and carbon monoxide (CO) leading to an activation of the cyclic guanosine monophosphate (cGMP) —protein kinase G (PKG) signalling. PPAR, peroxisome proliferator-activated receptor

to the lateral hypothalamus were described to regulate arousal and, thereby, sleep-wake behaviour.[32,33] Direct projections from the SCN to the ventrolateral preoptic nucleus (VLPO) are known. However, indirect SCN projections to the VLPO via, for example, the DMH and the medial preoptic area are thought to regulate sleep.[34] Additional SCN projections to the median preoptic nucleus (MPN) and the anteroventral paraventricular nucleus were described.[34,35] Furthermore, the SCN innervates the paraventricular nucleus of the hypothalamus and is thereby regulating several hormonal axes as well as melatonin release, which in turn feeds back to the SCN.[36,37] Incoming signals to the SCN originating from ipRGCs of the retina, that contain melanopsin, as well as metabolic stimuli integrate via the arcuate nucleus (ARC).[38,39] The mechanisms of communication within the circadian clock network of the body are still poorly understood. Factors involved are autonomic activation, body temperature, endocrine factors such as melatonin or glucocorticoids (GCs), but also behavioural functions such as the sleep-wake cycle or the timing of food intake.[20]

As mentioned above, other *Zeitgebers* than light have been described that are capable of affecting the circadian clock system, not all of which act through the SCN. The timed intake of food is a potent synchronizer of peripheral tissue circadian clocks while having little effect on the SCN itself.[40] Under extreme conditions, such as rest phase restricted feeding, this may lead to a complete uncoupling of central and peripheral clocks. It is believed that such internal chronodisruption may be one of the underlying reasons for the high prevalence of metabolic disorders in night shift workers.[41] On the other hand, recent work on rodents with disabled SCN function suggests that light may adjust clock gene transcription in peripheral tissues independent of SCN function.[42-44] The pathway of such peripheral photic resetting remains largely unclear, but direct autonomic activation or behavioural responses to external lighting conditions may play a role.

Considering the complexity of the circadian clock network with its millions of cellular timekeepers, the interaction of different oscillators in the coordination of overt circadian rhythms in behaviour and physiology and the temporal harmonization of different rhythms across the body

are important aspects of circadian timekeeping. The SCN is known to communicate with extra-SCN central nervous system (CNS) oscillators as well as with peripheral clocks (Figure 3).[45-47] Extra-SCN CNS oscillator output signals modulate neuronal functions such as mood and cognition but also communicate with peripheral tissue oscillators via induction of behavioural rhythms, for example, food intake, or neurohumoral rhythms.[46] Peripheral tissue clocks are then regulating physiological functions and rhythms such as energy metabolism.[46]

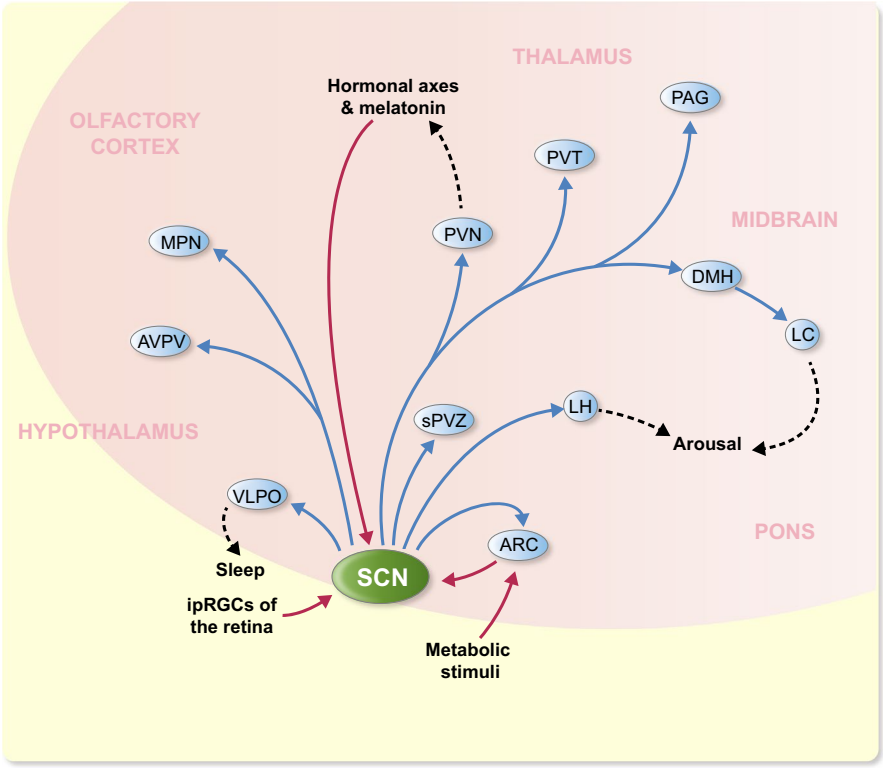

FIGURE 2 Suprachiasmatic nucleus (SCN) projections and incoming signals. Projections from the SCN mainly reach hypothalamic brain regions but also parts of the thalamus and midbrain (blue arrows). Incoming signals from intrinsically light-sensitive retinal ganglion cells (ipRGCs), the hormonal axes, melatonin, as well as metabolic stimuli are represented by orange arrows. ARC, arcuate nucleus; AVPV, anteroventral paraventricular nucleus; DMH, dorsomedial nucleus of the hypothalamus; LC, locus coeruleus; LH, lateral hypothalamus; MPN, median preoptic nucleus; PAG, periaqueductal grey; PVN, paraventricular nucleus; PVT, paraventricular nucleus of the thalamus; SCN, suprachiasmatic nucleus; sPVZ, subparaventricular zone; VLPO, ventrolateral preoptic nucleus

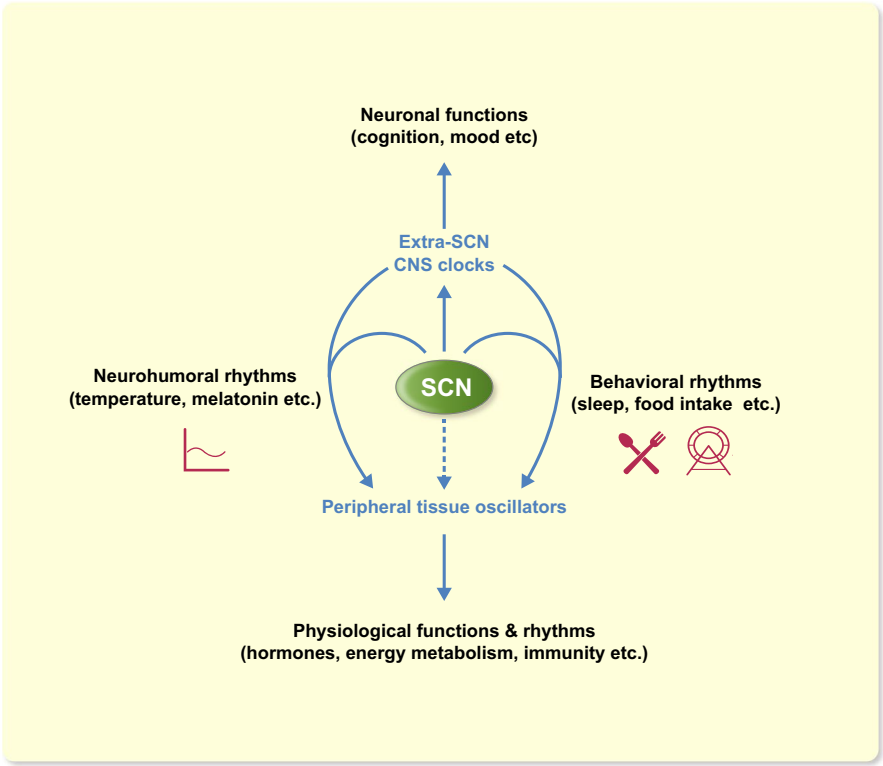

FIGURE 3 Clock network communication. The suprachiasmatic nucleus (SCN) synchronizes extra-SCN central nervous system (CNS) oscillators and peripheral tissue clocks. Extra-SCN CNS clocks, mostly dependent from SCN signals, regulate neuronal functions but also communicate with the periphery via neurohumoral and behavioural rhythms. Peripheral tissue clocks then influence physiological functions and rhythms. For details see text

Traditionally, the main power over temporal coordination had been assigned to the SCN and its entrainment by light. With the development of experimental tools to study clock function in specific tissues—for example, by tissue-specific gene targeting in mice—that view has been challenged. We know now that tissue clocks continue to measure time even when removed from the body and the main task of the SCN is the coordination of different tissue clocks with external time.[48] Moreover, tissue-specific ablation of clock function has been used to identify physiological roles for specific tissue clocks.[49] Ablation of clock function in liver cells, for example, impacts on glucose handling through regulation of glucose transporter expression in hepatocytes.[50] Loss of clock function in pancreatic beta cells also impacts on glucose metabolism by inhibiting insulin secretion and, thus, postprandial glucose disposal.[51,52] In the immune system, cell-type specific clocks have been implicated in cell migration and immune defences.[53,54] Most of these studies have focused on tissues and cells of the periphery while arguably the most visible outputs of the circadian clock—from sleep to appetite and temperature regulation—are functions of the CNS. Emerging data start to reveal the complex coordination of circadian timekeeping in the brain.

4 | METHODOLOGICAL APPROACHES

Different methodological approaches have been used to investigate circadian oscillators and their function. These questions were mostly assessed by lesion studies[55] and general[56] or conditional[57] genetic knockouts, but other techniques such as chemogenetics,[58] optogenetics[59] or clock rescue experiments in *Bmal1* deficient mice[60] were also used. All these approaches have their advantages and disadvantages or are used to focus on a certain strength, for example, specificity or efficiency.

Lesion studies have the advantage to compare, for example, task performance in the same mouse before and after the lesion and are widely used to investigate correlations between brain regions and behaviour. Lesions are achieved by the application of an electric current (electrolytic lesions), by neuron overstimulation with, for example, glutamate (excitotoxic lesions), or knife cuts. All these techniques have in common that they damage the whole tissue area and thereby also destroy its connectivity. Another option are tissue transplants which were used for studies of SCN function,[23] but have so far not broadly been applied to the characterization of extra-SCN brain oscillators.

A conventional genetic knockout would leave the tissue intact, but there is the risk of developmental effects as, for example, described for *Bmal1*-deficient mice.[61,62] Furthermore a global knockout is addressing gene function in the whole organism and not a certain tissue, which is achieved by conditional knockout. Viral approaches increase specificity, but often decrease efficiency compared to *Cre/loxP*-mediated conditional knockouts. The advantage of viral approaches is their potential for highly specific targeting of certain neuronal populations. Rescue experiments, for example, a rescue of *Bmal1* expression in *Bmal1*-deficient mice—either virally or via a *Cre* mouse line—are another approach to investigate the function of circadian oscillators. Viral tracing studies have been used to investigate the connections between the SCN and other brain regions such as the paraventricular nucleus and the medial prefrontal cortex and can be advantageous for testing the connectivity between different brain oscillators.[63-65]

Newer techniques such as chemo- or optogenetics allow for the functional characterization of specific neuronal populations in certain brain areas. While optogenetics allow a tight temporal control, intracranial transplants and repetitive stimulation are necessary to obtain effects in the circadian domain. Chemogenetics do not provide such a tight temporal control, but instead allow for long-time investigations with a single drug administration and without intracranial implants. It is, for example, possible to activate designer receptors exclusively activated by designer drugs (DREADDs) to directly manipulate circadian clock output.

Apart from the in-vivo studies there are several in-situ approaches to test clock function, for example, via gene or protein expression analyses. However, commercial antisera for several clock proteins are not always very specific, which might lead to problems in Western blots or immunohistochemical determinations. Explants from *Per2::LUC* mice were also used to study tissue circadian rhythms or to test for the effect of substances on tissue clock resetting. However, this method might not be ideal for neuronal population that cannot be isolated *en bloc*. Moreover, unwanted clock resetting during tissue preparation is possible.

5 | BRAIN CLOCKS OUTSIDE THE SCN

The retina was the first neuronal tissue outside the SCN to be shown to have endogenous, autonomous circadian rhythms of melatonin synthesis in vitro.[66] Since then, by studying rhythms in expression of clock genes and electrical activity, a variety of regions all across the brain spanning from tel- to metencephalon were found to oscillate.[45,67] Real-time bioluminescence recordings of clock gene reporters in different tissues allowed to investigate the sustainability of circadian oscillators when disconnected from the body. Of 27 different brain regions studied in mice by Abe et al, 14 showed rhythmicity beyond a single cycle. Most regions dampened fast indicating the dependency on pacemaker input.[68] With

the exception of very few extra-SCN brain regions, rhythmicity was lost after surgically or genetically ablating the SCN. These clocks were therefore called *secondary* or *slave* oscillators.[45]

The only other region besides the SCN to fulfil all characteristics of a circadian pacemaker is the olfactory bulb (OB). Research from Granados-Fuentes et al showed that the rhythmicity of the OB is autonomous, entrainable and temperature compensated.[69,70] Clock gene expression rhythms in the OB respond to light.[71] Moreover, odour was able to induce cFOS rhythms in OB and the associated piriform cortex. These rhythms persisted in SCN-lesioned mice, but bulbectomy also abolished rhythmicity in the piriform cortex.[72] Other cortical structures rhythmically express core clock components in a SCN-dependent manner in rats.[73,74] Interestingly, restricted-feeding induces rhythms in the cerebral cortex of SCN-ablated mice.[75] Cortical brain activity shows a diurnal pattern in humans and is speculated to be under GC-dependent circadian control.[76,77] In the prefrontal cortex, stress induces *Per1* and *cFos* mRNA expression independent of adrenalectomy in mice.[78] Gonadal hormones may play a role in this context.[79]

The hippocampus as a key structure of memory formation rhythmically expresses all core clock components.[80,81] These rhythms persist in constant darkness (DD) and in organotypic slice cultures for several days.[82] Interestingly, expression peak times correlate with the species' temporal niche.[82,83] Melatonin can reset hippocampal clocks[84] but pinealectomy does not markedly affect clock protein rhythmicity.[85]

The amygdala is another subcortical structure of the limbic system, which also shows robust clock protein expression rhythms depending on a functional SCN.[86] Amygdala clock rhythms are entrained by GCs,[87,88] but adrenalectomy abolishes rhythms exclusively in the central nucleus but not the basolateral amygdala.[86] Moreover, rhythms in the amygdala and the amygdala-associated bed nucleus of the stria terminalis (BNST) are also blunted after thyroid- and parathyroidectomy[89]—but not after pinealectomy.[85] Time-restricted feeding restores rhythms in the amygdala and BNST of adrenalectomized animals.[90] Moreover, clocks in both tissues are sensitive to sex steroids.[91]

The *nucleus accumbens* (NAc) and caudate putamen make up most of the striatum, a region coordinating multiple functions of cognition and motivation. The ex-vivo rhythmicity of the NAc is disrupted after mood manipulation in mice[92] and clock gene expression patterns are altered by a free-choice high-fat/high-sugar diet in rats.[93] Natsubori et al showed that putamen slices exhibit robust rhythmicity despite a lack of dopaminergic input,[94] which was previously hypothesized to be the major regulator of this brain clock.[95] It therefore remains to be shown, how exactly these striatal brain oscillators are entrained.

It is still unclear how exactly the lateral habenula (LHb) of the epithalamus keeps time.[96] An early study showed a circadian rhythm in firing rates in sync with the SCN and photic responsiveness of the habenula in rats,[97] while another study found no increase in cFOS expression by light in mice.[98] Sakhi et al proposed a more influential role of intrinsic signals compared to SCN-derived transmitters or visual information in the LHb.[99] The LHb, thus, seems to be rather autonomous and self-sustained brain clock: ex-vivo oscillations persist independent of action potentials[45] and even when prepared from SCN-ablated animals.[100] Still, SCN input is needed to keep two distinct clocks within the habenula in sync.[100] Again, obesogenic choice diets abolish rhythmicity in this region indicating a sensitivity to nutrient input.[101]

Many hypothalamic extra-SCN oscillators are particularly sensitive to the metabolic state or food timing. Rhythms detected in the dorsomedial, the lateral and ventromedial hypothalamus (VMH) are weak unless animals are kept under time-restricted feeding conditions.[102] The ARC is a critical region for the control of homeostasis. Despite robust rhythmicity in vitro,[103] the ARC loses clock gene oscillations when food is restricted to the daytime in nocturnal animals.[104] Genetic disruption of the SCN clock does not completely eliminate rhythmic dopamine release in the ARC indicating a semi-autonomous clock machinery.[105] Nevertheless, cutting the connections between ARC and the SCN results in desynchronization of the former.[106] Hypothalamic regions are important control centres of many rhythmic physiological functions. It is, therefore, not surprising that most areas show pronounced oscillations.[68,79] Midbrain oscillators are less well characterized, and evidence of rhythmicity is often vague. For example, slice cultures of the *substantia nigra* were deemed arrhythmic in one,[68] but rhythmic even in SCN-lesioned rats in another study.[94] Circadian gene expression is found in regions of the hindbrain and thalamus. For a thorough discussion see the recent review from Paul et al[67] Taken together, it becomes clear that circadian clocks are distributed throughout the brain. Most of these oscillators are strongly dependent on the SCN. However, some of these extra-SCN oscillators show a certain autonomy and appear sensitive to non-photic *Zeitgebers* (Figure 4).

6 | FUNCTIONAL STUDIES OF EXTRA-SCN OSCILLATORS

While, at least on the level of clock gene expression, 24-hour rhythms have been described across the whole brain (eg search for 'ARNTL' and select circadian times series data sets at https://gp3.mpg.de), for some of these regions, studies on the physiological significance of their clocks are just emerging. Considering the rising prevalence of

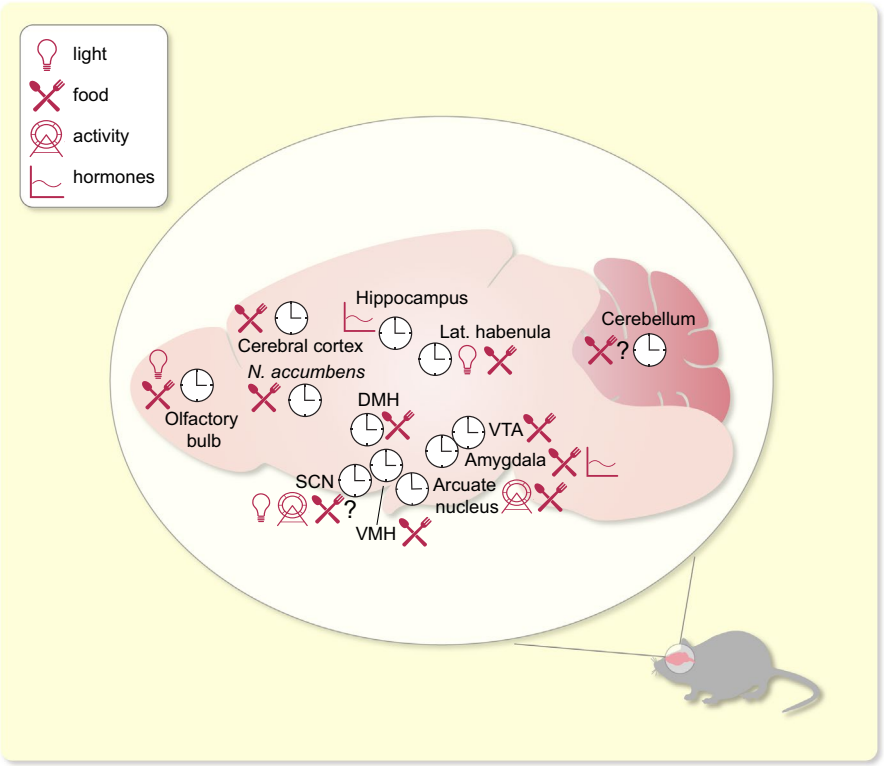

FIGURE 4 Regulation of extra-SCN oscillators in the brain. Reported resetting signals, light, food, activity or hormones, are illustrated as symbols next to the described oscillator. DMH, dorsomedial nucleus of the hypothalamus; Lat., lateral; N., nucleus; SCN, suprachiasmatic nucleus; VMH, ventromedial nucleus of the hypothalamus; VTA, ventral tegmental area

neuropsychiatric disorders and their strong correlation with circadian rhythm alterations, there is a strong interest both from scientific and clinical sides. We here summarize some of the studies aimed at elucidating functional aspects of known extra-SCN brain clocks and oscillators (Table 1).

As mentioned above the OB houses a robust clock with pacemaker-like qualities. However, its functional significance is still not well known. It was suggested to regulate 24-hour rhythms in olfaction via vasoactive intestinal polypeptide signalling modulating OB outputs.[107] Diurnal rhythms of α1-2-fucose in secondary olfactory neurons of the OB are dampened in clock gene mutant mice.[108] OB-gated olfaction can enhance photic resetting of the SCN.[109] The OB clock responds to feeding rhythms[110] but OB ablation in rats does not affect food-anticipatory activity (FAA)[111] nor running-wheel behaviour in general.[70] In hamsters, however, olfactory bulbectomy lengthens free-running period in line with its modulatory input to SCN resetting.[112]

Oscillators in the habenula were described in the rostral and caudal part[113] as well as in the medial part of the LHb.[114] LHb slices show a circadian rhythm in firing rates peaking in the late subjective light phase and depending on a functional molecular clock.[99,100] Since presynaptic potentiation of ventral tegmental area (VTA)-projecting neurons of the LHb was reported in learned helplessness models of depression,[115] a functional temporal influence of LHb rhythms on monoamine release and the reward system was suggested.[113,116] Furthermore, a possible influence of the LHb clock on hedonic food intake was suggested because of absent day-night activity of PER2 in a mouse model of diet-induced obesity[117] and a negative regulation of hedonic food intake by glutamatergic projections from the lateral hypothalamic area to the LHb.[118] The LHb clock was further discussed in context of mood alternations and the regulation of sleep homeostasis.[119,120] Clock gene expression levels in the LHb are affected by chronic mild stress.[119] Sleep deprivation alters clock gene expression in the LHb and, interestingly, LHb-lesioned rats show altered sleep-wake rhythms with increased slow-wave sleep.[120]

Clock-deficient *Cry1/2*$^{-/-}$ mice show depression-like behaviour associated with alterations in neuronal growth factor expression in the basolateral amygdala, suggesting that the clock is involved in amygdala functioning to modulate emotional states.[121] Somatostatin mRNA in the amygdala is rhythmic and somatostatin-deficient mice lose circadian rhythms in anxiety-related behaviour indicating that the amygdala oscillator may be involved in the circadian modulation of anxiety.[122]

The ARC is a main hub for the central integration of metabolic signals,[26] whereby two neuronal populations are important: pro-opiomelanocortin neurons inhibit, whereas neuropeptide Y/agouti-related protein neurons increase appetite.[123,124] Therefore, many studies investigated the effect of timing of food intake on the ARC oscillator. Clock gene expression in the ARC responds to meal timing.[104,125,126] Rhythmic clock gene expression in neuroendocrine dopaminergic neurons of the ARC suggested that prolactin secretion might be under the control of the ARC oscillator.[127]

TABLE 1 Suggested functional outputs of extra-SCN brain oscillators

Extra-SCN oscillator	Suggested function	Experimental evidence[a]	Reference(s)
Olfactory bulb (OB)	Olfaction	Expression studies	107
	SCN photic resetting	Expression studies	109
		Lesion studies[a]	
Lateral habenula (LHb)	Monoamine release	Electrophysiology	116
	Reward	Expression studies	101,113
		Electrophysiology	
	Food intake	Expression studies	101,117
		Lesion studies[a]	
	Mood	Expression studies	119
	Sleep homeostasis	Expression studies	120
Amygdala	Emotion, anxiety	Expression studies,	121,122
		Neuropeptide manipulation[a]	
Arcuate nucleus (ARC)	Prolactin secretion	Expression studies	127
	Body temperature	Electrophysiology	26
	Sleep-wake cycle	Neuropeptide manipulation[a]	129
Dorsomedial hypothalamus (DMH)	Unknown (food anticipation discussed)	Lesion, Tissue clock manipulation[a]	55,102,130,131
Ventromedial hypothalamus (VMH)	Food anticipation	Expression studies,	137
		Lesion studies[a]	
	Energy expenditure & thermogenesis	Tissue clock manipulation[a]	140
Ventral tegmental area (VTA)	Reward	Expression studies	141,142
		Clock gene manipulation	
	Locomotor activity	Tissue clock manipulation[a]	144
	Anxiety & depression	Tissue clock manipulation[a]	144
Nucleus accumbens (NAc)	Stress response and anxiety	Clock gene manipulation,	148
		Expression studies	
	Reward	Clock gene manipulation,	150,151
		Expression studies	
	Food anticipation	Expression studies	152,153
Hippocampus	Memory formation	Clock gene manipulation	154
	Depression	Expression studies	158
Cerebral cortex	Mood	Tissue clock manipulation[a]	57
	Food anticipation	Expression studies	160
	Jetlag regulation	Expression studies	161
Cerebellum	Unknown (food anticipation discussed)	Expression studies, Lesion studies[a]	162,163

[a]In-vivo functional manipulation of tissue, tissue clock or tissue output.

Interestingly, rats with ablated SCN-ARC connections show arrhythmic GC levels, body temperature and locomotor activity in DD suggest a close interaction in SCN and ARC clocks in the regulation of these outputs.[106] Guzmán-Ruiz et al reported that the balance of α-MSH release from the ARC together with arginine vasopressin neurons from the SCN is fundamental for the daily body temperature rhythm through projections to the MPN.[128] Padilla et al investigated the effect of kisspeptin-expressing neurons in the ARC ($Kiss1^{ARH}$) on locomotor activity, body temperature, sleep and food intake rhythms in female mice. Toxin-silencing of $Kiss1^{ARH}$ neurons leads to arrhythmic feeding patterns and increased

body weight, but not food intake. Furthermore, daily activity is decreased in these mice, body temperature and locomotor activity rhythms become unstable along with alterations in the sleep-wake cycle. Data were gained from female mice but changes in locomotor activity rhythms and body weight were shown to be independent of ovarian estrogen.[129]

The DMH was controversially discussed to be the side of the food-entrainable oscillator (FEO).[55,102,130,131] In particular, Fuller et al suggested a clock gene-based FEO within the DMH.[132] However, this study was partially refuted.[133,134] Retrograde tracing and DMH lesions suggest a neuronal network involving DMH-SCN interactions as likely source for FAA.[135] Studies with DMH-ablated mice suggest that clock gene expression in the DMH is sensitive to food intake but the DMH oscillator is not essential for FAA and temperature rhythms in mice.[125] Therefore, the function of the DMH oscillator remains to be shown.

Even if involved in synchronizing feeding rhythms, SCN- and VMH lesion experiments show that the VMH itself does not contain a self-sustained oscillator controlling feeding rhythms.[136] It may, however, be involved in FAA. Although VMH nucleus lesions do not abrogate FAA, they changed their development under restricted feeding conditions.[137] VMH lesions were also shown to influence temperature and locomotor activity rhythms in food-restricted rats.[138] By using mice with an ablation of *Sirtuin (Sirt1)* in steroidogenic factor 1 (SF1) neurons in the VMH it was shown that SIRT1 links nutritional signals to the circadian clock.[139] Targeted deletion of the essential clock gene, *Bmal1*, within these neurons alters circadian energy expenditure rhythms via brown adipose tissue thermogenesis.[140]

Disturbed clock gene expression in reward-related brain regions such as the VTA after chronic cocaine application suggests a role of the VTA clock in the regulation of reward pathways.[141] In *Per2* clock gene mutant mice, altered monoamine oxidase (*Maoa*) mRNA expression levels as well as reduced MAOA is accompanied by elevated dopamine levels in the NAc.[142] Diurnal rhythms of tyrosine hydroxylase in the VTA are regulated by NAD+-dependent activity of SIRT1.[143] VTA specific clock knockdown leads to hyperactivity, reduced anxiety-related and increased depression-like behaviour.[144] A study with 6-hydroxydopamine-induced VTA lesions in rats suggests that the mesolimbic dopaminergic system is involved in regulating circadian drinking and locomotor activity rhythms.[145] While most neurons in the VTA are either dopaminergic or GABAergic, Luo et al described an additional non-dopaminergic, non-GABAergic neuronal population within the VTA. These cells selectively fire throughout the active phase and are most likely projecting to the hippocampus suggesting a second non-dopaminergic circadian function of the VTA oscillator.[146]

Unpredictable chronic mild stress models influence the rhythms of clock gene expression in the *nucleus accumbens* (NAc) in anhedonic rats.[119,147] Specific knock-down of *Per1* and *Per2* in the NAc by RNA interference increases anxiety-like behaviour.[148] Knock-down of another clock gene, *Rev-erbα*, in the NAc modulates anxiety-related behaviour and sociability in female but not in male mice.[149] Diurnal rhythms in dopamine receptor D3 expression suggest an involvement in reward processing, especially since cocaine disrupts both NAc clock gene as D3 expression rhythms in this area.[150,151] Along this line, an involvement of NAc clocks in FAA and food entrainment was suggested.[152,153]

Rev-erbα knockout mice show abnormal early long-term potentiation (LTP) in the hippocampus specifically during the subjective night.[56] Several clock gene mutant mice show abnormal LTP and deficits in learned behaviour.[82,154] Circadian reactivation of MAPK and cyclic adenosine monophosphate (cAMP) was suggested to influence long-term memory formation.[155] Phosphorylation of cAMP responsive element-binding protein (CREB) is rhythmic in hippocampal slices.[156,157] Chronic unpredictable stress persistently changes clock protein levels in the hippocampus suggesting that the hippocampal oscillator might also have a role in depressive-like behaviour.[158] Finally, hippocampal neurons from *Per1* mutant mice have a depressed autophagic machinery which might increase vulnerability during cerebral ischaemia.[159]

A conditional knockout of *Bmal1* in neurons of the cerebral cortex was shown to influence behaviour and mood (depressive-like state) and reduced noradrenaline levels.[57] Protein kinase C gamma (PKCγ)-mediated stabilization of cerebral BMAL1 levels was shown to affect food anticipation[160] and depletion of noradrenergic innervation from the LC in *Ear2* mutant mice results in dampened cortical clock gene rhythms and alterations in photic entrainment under experimental jetlag conditions.[161] Studies in mice with the hotfoot mutation suggested cerebellar oscillators to be involved in food anticipation.[162] Targeted deletion of *Bmal1* in the granular layer of the cerebellum, however, had a strong influence on the cerebellar clock gene oscillations, but did not influence food anticipation.[163]

7 | CONCLUSION

In summary, despite the primary focus of the circadian field on deciphering the various functions of the SCN pacemaker for some decades, there is now a *plethora* of studies describing the involvement of different extra-SCN central oscillators in various physiological functions such as activity rhythms, appetite, memory formation and mood. Nevertheless, considering the broad impact of circadian disruption on cognitive and neuropsychiatric functions, there is a clear interest from both scientists and clinicians to further dissect the regulation of specific brain oscillators and their contribution to physiology and disease. Still, many brain

clocks remain to be described. With new genetic tools such as virus-mediated gene editing or chemogenetics more specific targeting of neuronal subpopulations becomes possible in the circadian context. Such studies will without doubt help us to better understand circadian network regulation across the brain and potentially devise novel therapeutic avenues for the treatment of common brain disorders such as major depression or neurodegeneration.

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CONFLICT OF INTEREST

The authors declare no competing interests.

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