Circuit development in the master clock network of mammals

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Circuit development in the master clock network of mammals

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Abstract  
Daily rhythms are generated by the circadian timekeeping system, which is orchestrated by the master circadian clock in the suprachiasmatic nucleus (SCN) of mammals. Circadian timekeeping is endogenous and does not require exposure to external cues during development. Nevertheless, the circadian system is not fully formed at birth in many mammalian species and it is important to
understand how SCN development can affect the function of the circadian system in adulthood. The purpose of the current review is to discuss the ontogeny of cellular and circuit function in the SCN, with a focus on work performed in model rodent species (i.e., mouse, rat, and hamster). Particular emphasis is placed on the spatial and temporal patterns of SCN development that may contribute to the function of the master clock during adulthood. Additional work aimed at decoding the mechanisms that guide circadian development is expected to provide a solid foundation upon which to better understand the sources and factors contributing to aberrant maturation of clock function.

Abbreviations
ALDH1  Aldehyde Dehydrogenase 1 Family Member L1
APC  Adenomatous Polyposis Coli
ASCL  Achaete-Scute Family Transcription Factor
AVP  Arginine vasopressin
BB2  Bombesin 2 receptor
BCL-2  B-Cell lymphoma 2 protein
bHLH  Basic helix-loop-helix
Bmal1/BMAL1  Brain and muscle Arnt-like Protein 1 gene/protein
BrdU  5-Bromo-2‘-deoxyuridine
CB  Calbindin
Clock/CLOCK  Circadian locomotor output cycles Kaput gene/protein
CR  Calretinin
Cry/CRY  Cryptochrome gene/protein
DLX  Distal-less homeobox
E#  Embryonic day
FGF  Fibroblast growth factor
FOXD  Forkhead box domain
FZD  The Wingless receptor, Frizzled
GABA  γ-Aminobutyric acid
GAD  Glutamic acid decarboxylase
GFAP  Glial fibrillary acidic protein
GP  Gestational period
GRP  Gastrin-releasing peptide
HES  Hairy enhancer of split
ipRGCs  Intrinsically sensitive retinal ganglion cells
L1  L1 protein
LHX  LIM homeobox
MAPK  Mitogen-activated protein kinase
MBP  Myelin basic protein
NCAM  Neural cell adhesion molecule
NeuN  Neuronal nuclei protein
NK-1  Substance P receptor
NKX  Nkx homeobox
NMS  Neuromedin S
P#  Postnatal day
Per/PER  Period gene/protein
1 INTRODUCTION
The circadian system generates daily rhythms that anticipate changes in the environment caused by the Earth's rotation (Hut & Beersma, 2011; Pittendrigh, 1960). The master circadian clock in mammals is located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus (Weaver, 1998), which receives photic cues and provides daily signals to downstream tissues to coordinate the timing of overt rhythms (Figure 1a; Mohawk, Green, & Takahashi, 2012). Daily rhythms are endogenously generated and not “learned” through exposure to external cues. Nevertheless, evidence from a variety of mammalian species suggests that the circadian system undergoes postnatal maturation much like the rest of the brain. Human newborns start to display daily rhythms gradually over the first few months of life, with body temperature rhythms developing in the first week after birth, sleep:wake rhythms emerging in the first 1–2 months, and hormone rhythms appearing around 3 months of age (Kennaway, Goble, & Stamp, 1996; Kleitman & Engelmann, 1953; McGraw, Hoffmann, Harker, & Herman, 1999; Price, Close, & Fielding, 1983; Rivkees, 2007). Furthermore, circadian development can be affected by perinatal environmental conditions, which can influence health outcomes. For instance, 24/7 lighting in Neonatal Intensive Care Units decreases weight gain in babies born prematurely, increases latency to discharge from the hospital, and delays the timing of sleep:wake rhythm consolidation (Brandon, Holditch-Davis, & Belyea, 2002; Mann, Haddow, Stokes, Goodley, & Rutter, 1986; Vasquez-Ruiz, et al., 2014). In addition, season of birth is associated with individual differences in chronotype (Mongrain, Paquet, & Dumont, 2006; Natale & Di Milia, 2011), which can increase the risk of developing metabolic and mood disorders in adulthood (Erren, Koch, Gross, Reiter, & Meyer-Rochow, 2012; Merikanto et al., 2013; Tonetti, Fabbri, Martoni, & Natale, 2012; Yu et al., 2015). These studies indicate that the circadian system is sensitive during the perinatal period, and
underscore the importance of understanding how development of the circadian system affects its operation in adulthood. In this review, we describe SCN development, with a focus on work performed in model rodent species (i.e., mouse, rat, and hamster). Particular emphasis is placed on the spatial and temporal patterns of SCN development at the cellular and network level that may contribute to the function of the master clock during adulthood.

Figure 1 Development of the circadian timekeeping system. (a) In adulthood, the suprachiasmatic nucleus (SCN) receives light input via the retinohypothalamic tract and provides daily outputs signals to downstream tissues to coordinate the timing of overt rhythms. (b) Simplified model of the circadian molecular clock in mammals, which is composed of self-sustained transcriptional–translational feedback loops that regulate daily expression of clock genes and their protein products. In SCN neurons, the transcription factors CLOCK and BMAL1 dimerize and activate Period (Per) and Cryptochrome (Cry) expression during the day. After translation, PER and CRY dimers repress their own transcription at night. This core feedback loop is interlocked with other transcriptional loops that stabilize and augment circadian function. For example, REV-ERB and ROR regulate the daily expression of Bmal1. (c) The shell-core model of the adult SCN network illustrating the spatial location of five neuronal subclasses in mice. AVP: Arginine Vasopressin; CB: Calbindin; CR: Calretinin; GRP: Gastrin-Releasing Peptide; VIP: Vasoactive Intestinal Polypeptide. Note: all five peptides are also expressed in rats and hamsters, although the expression of CB and CR differs among rodent species. (d) Timeline of SCN development. Important milestones are illustrated for SCN development in rodents. Most information illustrated on the timeline derives from studies using mice, but detailed information on the timing of foetal metabolic/electrical rhythms, Rev-erb rhythms, synaptogenesis, and glial maturation are only available for rats. Early genetic markers of SCN differentiation are labelled purple to represent those for which effects on SCN development have been reported.

In adulthood, the SCN is a network of cellular clocks that display daily rhythms in cellular physiology, including metabolism, electrical activity, gene/protein expression, and peptide release (Hastings, Maywood, & Brancaccio, 2018). Circadian rhythms are generated at the cellular level by self-sustained transcriptional–translational feedback loops that regulate daily expression of clock genes and their protein products (Figure 1b; Takahashi, 2017). Briefly, the transcription factors CLOCK and BMAL1 dimerize to activate expression of Period (Per1, Per2) and Cryptochrome (Cry1, Cry2) genes, which are translated into protein products that repress their own transcription. This core feedback loop is interlocked with other transcriptional loops that stabilize and augment circadian function (Figure 1b; Takahashi, 2017). This molecular clock operates in a heterogeneous population of SCN cells that
communicate with one another to function as a unit. A majority of SCN neurons express the transmitter GABA, but they can be classified into subclasses based on neuropeptide expression (Abrahamson & Moore, 2001; Moore & Speh, 1993). Classic models organize the SCN into two complementary compartments, known as the shell and the core, which are often distinguished using arginine vasopressin (AVP) in the SCN shell and vasoactive intestinal polypeptide (VIP) in the SCN core (Figure 1c; Antle, Foley, Foley & Silver, 2003; Moore, Speh, & Leak, 2002). In general, this organizational scheme is consistent across many mammalian species, (Cassone, Speh, Card, & Moore, 1988; Stopa, King, Lydic, & Schoene, 1984) although it is a simplified model (Morin, 2007). In addition to neurochemical differences, SCN neurons in the shell and core regions differ in clock function and photic responses (Antle et al., 2003; Hastings et al., 2018), which may be established during network development under the guidance of cell-intrinsic and/or cell-extrinsic factors. Development of the circadian system has been covered in several excellent reviews published recently (Bedont & Blackshaw, 2015; Landgraf, Koch & Oster, 2014; Sumova, Sladek, Polidarova, Novakova & Houdek, 2012), and the current review seeks to provide an update on recent progress made in this area (Figure 1d, see also Honma review, same issue).

2 SCN CELLULAR DEVELOPMENT
The SCN is located within the ventral subdivision of the anterior hypothalamus. Similar to other parts of the brain, cellular development in the hypothalamus proceeds along several stages including proliferation and differentiation. Initial stages of cellular development in the rodent hypothalamus are largely completed during gestation, but latter stages can continue after birth. Embryonic age in rodents is defined typically by the presence of a vaginal plug on the morning after fertilization, which is labelled embryonic day 0.5 (E0.5) or embryonic day 1 (E1) depending on the conventions adopted by the laboratory. Because rodent models differ in the duration of gestation (i.e., mouse: 20 days, rat: 21–23 days, and hamster: 16 days), species differences in the timing and patterning of SCN development are considered below.

2.1 Hypothalamic development
The progenitor cells that give rise to the hypothalamus are located in the matrix layer surrounding the third ventricle (Bedont & Blackshaw, 2015). Hypothalamic development generally follows an outside-in pattern where neurons born early are displaced outward by neurons born later (Altman & Bayer, 1978; Ifft, 1972; Shimada & Nakamura, 1973), although this model may not apply to all hypothalamic regions (Markakis & Swanson, 1997; Padilla, Carmody & Zeltser, 2010). In general, the anatomy and development of the hypothalamus is fairly well conserved across vertebrate species (Ware, Hamdi-Roze & Dupe, 2014; Xie & Dorsky, 2017). Furthermore, the timing of hypothalamic neurogenesis is fairly consistent across rodent species when embryonic age is normalized to the duration of the gestational period (GP). For instance, hypothalamic neurogenesis occurs in mice between E10 and E16 (50%–80% GP) and in rats between E11 and E19 (50%–85% GP).

During early stages of development, signals secreted by the ventral floor plate pattern the hypothalamus into discrete regions (Bedont & Blackshaw, 2015). Sonic hedgehog (encoded by Shh) is a lipid-linked polypeptide signal released from the ventral floor plate that is necessary for induction of the hypothalamus and drives Shh expression in the anterior hypothalamus. In mice, Shh is expressed by the anterior hypothalamus between E8.5 and E10.5 (Alvarez-Bolado, Paul, & Blaess, 2012). Deletion
of Shh from the basal hypothalamus eliminates expression of early genetic markers of the ventral anterior hypothalamus (Shimogori et al., 2010), including members of the LIM homeobox family (Lhx). In addition to SHH, the ventral floor plate releases other secreted factors that contribute to hypothalamic patterning, such as modulators of Wingless/Integrated (WNT) signalling (Bedont & Blackshaw, 2015; Rowitch & Kriegstein, 2010). These morphogenic cues can modulate several important processes during hypothalamic induction, including neurogenesis, differentiation, and axon guidance.

During neurogenesis, the ventral anterior hypothalamus expresses a variety of genetic markers, including Lhx2, Retinal and anterior neural fold homeobox (Rax), Forkhead domain 1 (Foxd1), and NK2 homeobox 2 (Nkx2.2). The expression of these early markers is transient, starting before the onset of neurogenesis and ending with its conclusion (Bedont & Blackshaw, 2015; Newman et al., 2018; Shimogori et al., 2010). For example, Rax is expressed in the ventral hypothalamus of the mouse between E10.5 and E14.5 (Pak, Yoo, Miranda-Angulo, Wang, & Blackshaw, 2014). Deletion of Rax prior to E8.5 disrupts patterning of the mediobasal hypothalamus (Orquera, Nasif, Low, Rubinstein, & de Souza, 2016), but later deletion causes aberrant rewiring in a specific progenitor pool (Lu et al., 2013). These observations suggest that early genetic markers are critical for the patterning of the hypothalamus and play more specific roles during later stages by affecting differentiation. Key events regulated by early genetic programmes include the activation of additional transcription factors, such as Ventral anterior homeobox 1 (Vax1), Six homeobox 3 (Six3), and Six6 (Hallonet, Hollemann, Pieler, & Gruss, 1999; Jean, Bernier, & Gruss, 1999; Lagutin et al., 2003; Li, Perissi, Liu, Rose, & Rosenfeld, 2002), which are often retained after differentiation to maintain cell-type specificity. The SCN is similar to other regions of the hypothalamus in its early expression of many of the genes described above, and the influence of these genetic programmes on SCN development is discussed in the following sections.

2.2 SCN neurogenesis

The bulk of SCN neurogenesis occurs over a 3- to 4-day interval corresponding to 60%–80% GP in mice, rats, and hamsters (Altman & Bayer, 1978; Davis, Boada, & LeDeaux, 1990; Kabrita & Davis, 2008). In the mouse, SCN neurogenesis occurs during E11.5–15.5 (58%–78% GP), with a peak at E13.5 (Kabrita & Davis, 2008; Shimada & Nakamura, 1973). Similarly, SCN neurogenesis occurs between E13.5 and E17 (58%–75% GP) in rats (Altman & Bayer, 1978; Ifft, 1972), and between E9.5 and E13.5 (60%–84% GP) in hamsters (Crossland & Uchwat, 1982; Davis et al., 1990). Following the interval of neurogenesis, the SCN is clearly defined as a cluster of densely packed neurons with small soma (Moore & Bernstein, 1989).

Prior to SCN neurogenesis, Lhx2, Rax, Foxd1, and Nkx2.2 are expressed in the mouse hypothalamus, but these markers are absent after SCN neurogenesis is complete at E16 (Ferran, Puelles, & Rubenstein, 2015; Shimogori et al., 2010; Vandunk, Hunter, & Gray, 2011). As in other hypothalamic regions, morphogen signalling is thought to influence SCN neurogenesis and development. Consistent with this idea, the WNT receptor Frizzled 5 (Fzd5) is detected at E10.5–13.5 specifically in cells that lack post-mitotic markers (Vandunk et al., 2011). To our knowledge, the contribution of Fzd5 to SCN development has not been examined directly, but represents an interesting area for future work given its role in other circuits of the brain (Liu, Wang, Smallwood, & Nathans, 2008; Sarin et al., 2018). In addition, members of the Fibroblast growth factor (Fgf) family are expressed in the ventral anterior
hypothalamus of the mouse at E11.5 (Ferran et al., 2015). Loss of Fgf signalling has been shown to reduce VIP expression and SCN volume at P0 (Miller, Kavanaugh, & Tsai, 2016), but consequences for circadian behaviour after birth have not been explored.

The spatial patterning of neurogenesis across the ventrodorsal axis is consistent across rodent species, with SCN neurons in the ventral core of the network having an earlier birthdate than their counterparts in the dorsal shell (Altman & Bayer, 1978; Davis et al., 1990; Kabrita & Davis, 2008). In the mouse, SCN core neurons are born first (peak at E12, 60% GP), and SCN shell neurons are born at a later age (peak at E13.5, 68% GP) corresponding to the peak of proliferation in AVP neurons at E14 (Kabrita & Davis, 2008; Okamura et al., 1983). Similar ventral-to-dorsal patterning occurs in rats and hamsters (Altan & Bayer, 1978; Davis et al., 1990). The neurogenesis of specific types of SCN neurons has been examined most extensively in the hamster (Antle, LeSauter, & Silver, 2005). At E10.5–E11.5 (66%–72% GP), neurogenesis occurs in SCN core neurons that later express VIP, Gastrin-Releasing Peptide (GRP), Substance P, or Calbindin. In contrast, genesis of AVP neurons occurs over a wider timeframe that extends into later embryonic ages (peak E10.5–E12.5, 66%–78% GP). It has been suggested that SCN core and shell neurons derive from distinct progenitor pools in the neuroepithelium (Altman & Bayer, 1986), but the precise mechanisms that regulate cell-type differences in the timing of SCN neurogenesis are not well understood. On one hand, it is possible that cell fate is determined by intrinsic programmes operating in neuronal precursors derived from distinct pools of progenitor cells. Another possibility is that extrinsic cues present in the microenvironment determine the maturation of SCN neurons proliferating at different times from the same progenitor pool. Which of these two models is most accurate for SCN development remains an open question, but both intrinsic and extrinsic factors have been shown to influence differentiation of GABA neurons in other brain regions (Quattrocolo, Fishell, & Petros, 2017).

There is also a spatial gradient of neurogenesis across the anteroposterior SCN, where the duration of neurogenesis is longer and ends later in the anterior SCN (Altman & Bayer, 1986; Davis et al., 1990; Kabrita & Davis, 2008). These studies indicate that SCN shell neurons are produced in at least two neurogenic waves, with those in the middle-posterior regions generated before those in the anterior pole. A similar posterior-to-anterior patterning of the SCN shell has been detected in mouse, hamster, and rat SCN (Altman & Bayer, 1986; Antle, LeSauter & Silver, 2005; Davis et al., 1990; Kabrita & Davis, 2008), although the opposite gradient was reported initially in the rat (Altman & Bayer, 1978). In addition, work suggests that there may be sex- and/or hormonally driven differences in the timing of neurogenesis across this axis (Abizaid, Mezei, Sotonyi, & Horvath, 2004). Anteroposterior patterning of SCN neurogenesis is of potential interest because photoperiodic encoding occurs along this axis in adulthood (Hazlerigg, Ebling, & Johnston, 2005; Inagaki, Honma, Ono, Tanahashi, & Honma, 2007). Thus, developmental patterning along the anteroposterior axis may influence photoperiodic modulation of behaviour and physiology later in life.

The large majority of SCN neurogenesis is complete during gestation, but the number of SCN neurons increases by 50% over the first 2–5 days after birth (Ahern et al., 2013; Muller & Torrealba, 1998). Consistent with these findings, a low rate of cytogenesis in the rat SCN has been observed in the first week after birth (Seress, 1985). Furthermore, neurogenesis has been noted to occur in the anterior SCN during puberty and adulthood in female rats using 5-bromo-2′-deoxyuridine (BrdU) to label
proliferating neurons (Mohr, DonCarlos & Sisk, 2017), suggesting that the SCN may be an additional neurogenic niche in hypothalamus of adult mammals (Yoo & Blackshaw, 2018). Although BrdU can produce false positives (Duque & Rakic, 2011), inhibiting cell proliferation decreases adult SCN neurogenesis and delays the timing of the luteinizing surge (Mohr et al., 2017). Because reproductive rhythms are regulated by the SCN (Williams & Kriegsfeld, 2012), this raises the possibility that SCN neurons proliferating during adulthood may integrate into clock circuits to influence circadian function. The potential for adult SCN neurogenesis is especially interesting given the unique transcriptional profile displayed by adult SCN neurons, including low levels of NeuN immunoreactivity (Morin, Hefton & Studholme, 2011) and continued expression of genes more commonly detected in pluripotent progenitor cells (Beligala, De & Geusz, 2018; Brown et al., 2017; Hoefflin & Carter, 2014; Saaltink, Havik, Verissimo, Lucassen & Vreugdenhil, 2012; Sato et al., 2011; Vandunk et al., 2011). Given this pattern of results, it would be interesting to test the potential role of these factors in the adult SCN and whether their expression is modulated by environmental factors that influence clock function (e.g., light).

2.3 Early stages of SCN differentiation

Early differentiation requires transcriptional changes that serve to restrict pluripotency and specify cell fate (Achim, Salminen, & Partanen, 2014). One of the most well-studied pathways contributing to this process involves Notch signalling (Ware et al., 2014). Notch signalling maintains progenitor cell pools and activates self-renewal genes, such as *Hairy enhancer of split (Hes)*, which is a class E basic helix-loop-helix (bHLH) transcription factor that inhibits differentiation by suppressing the expression of pro-neuronal genes. Inactivation of Notch signalling antagonizes factors related to progenitor cell identity (e.g., *SoxB1*), causes exit from the cell cycle, and leads to up-regulation of pro-neuronal bHLH transcription factors in neuronal precursors. One such pro-neuronal factor is *Achaete-Scute Family bHLH Transcription Factor 1 (Ascl1, also known as Mash1)*, which is expressed in precursors of hypothalamic GABA neurons between E11.5 and 13.5 (Allen Brain Atlas). Among its many gene targets, *Ascl1* activates expression of *Distal-less homeobox 1 (Dlx)* and *Dlx2* (Castro et al., 2011; Poitras, Ghanem, Hatch & Ekker, 2007), which are selector genes important for GABA neuron development (Plaat et al., 2018). *Dlx2* is expressed in the developing SCN of the mouse between E11.5 and E14.5 (Vandunk et al., 2011), which is consistent with GABA synthesis in adult SCN neurons (Abrahamson & Moore, 2001; Moore & Speh, 1993).

During early stages of differentiation, the SCN expresses several genetic markers also found in other hypothalamic nuclei. As mentioned earlier, *Lhx2, Rax, Foxd1*, and *Nkx2.2* are absent after the completion of SCN neurogenesis at E16 (Ferran et al., 2015; Shimogori et al., 2010; Vandunk et al., 2011). Despite being temporally restricted in expression, these genes activate genetic programmes important for later stages of SCN development. For instance, *Foxd1* is broadly expressed in the developing hypothalamus at E11.5, but loss of *Foxd1* function causes developmental defects in the anterior hypothalamus that appear after *Foxd1* levels have declined (Newman et al., 2018). SCN development is severely disrupted in *Foxd1*-deficient mice, with reduced expression of many genetic markers, loss of nuclear organization, and agenesis by P0.5 (Newman et al., 2018). The exact mechanisms by which the loss of *Foxd1* leads to delayed defects in SCN development remain unknown, and consequences for clock function are difficult to assess because *Foxd1*-deficient mice die within 24 hr after birth (Hatini, Huh, Herzlinger, Soares & Lai, 1996; Newman et al., 2018). But *Foxd1*-deficient
mice display reduced expression of Vax1 and Six3 at E12.5 (Newman et al., 2018), indicating that early genetic programming is disrupted. Vax1, Six3, and Six6 are expressed diffusely across the ventral anterior hypothalamus in mice from E11.5 to 14.5, but the expression of these genes becomes more restricted to the SCN region by E15.5 to 16.5 (Ferran et al., 2015; Newman et al., 2018; Shimogori et al., 2010; Vandunk et al., 2011). Consistent with developmental defects evident in Foxd1-deficient mice, loss of Vax1, Six3, or Six6 disrupts SCN differentiation and circadian control of behaviour later in life (Clark et al., 2013; Hoffmann et al., 2016; Vandunk et al., 2011). Collectively, this work establishes that early hypothalamic markers are necessary for normal SCN development, likely by controlling the activation of genetic programmes important for its later specification and maintenance.

The first selective SCN marker in the mouse is Lhx1, which is expressed throughout the SCN at E11.5 and becomes restricted to the central SCN by P0 (Vandunk et al., 2011). Earlier transcriptional activators are necessary for Lhx1 expression because loss of Lhx2, Foxd1, or Six3 will disrupt its normal patterning during SCN development (Newman et al., 2018; Roy et al., 2013; Vandunk et al., 2011). Lhx1 directly activates transcription of Vip (Hatori et al., 2014), and Lhx1 binding occurs at many other genes with enriched SCN expression (Bedont et al., 2014). In line with this, loss of Lhx1 in the ventral anterior hypothalamus reduces SCN levels of several neuropeptides known to be important regulators of master clock function, such as Vip, Avp, Prokineticin 2 (Prok2), and Grp (Bedont et al., 2014; Hatori et al., 2014). In addition, Lhx1-deficient mice display a 40% loss of SCN neurons by P4 due to increased cell death over the first few days after birth (Bedont et al., 2014). Consistent with reduced neuronal complement and neuropeptide expression, loss of Lhx1 disrupts SCN molecular and electrical rhythms by attenuating phase coherence among SCN neurons (Bedont et al., 2014; Hatori et al., 2014). Lastly, Lhx1-deficient mice display pronounced changes in circadian behaviour, with loss of daily rhythms under free-running conditions, altered responses to photic stimuli, and changes in sleep (Bedont et al., 2014, 2017; Hatori et al., 2014). Collectively, these studies reveal that the early activation of Lhx1 is critical for the proper development of the SCN network, and that its loss results in a failure to maintain terminal differentiation and prevent death of SCN neurons after birth.

A number of late genetic markers are expressed at E13.5 or later, including Zinc finger homeobox 3 (Zfhx3), POU Class 2 homeobox (Pou2f2, also known as Oct2), and the clock genes RAR-related orphan receptor a (Rora) and Rorb (Rivkees, Weaver & Reppert, 1992; Vandunk et al., 2011). Recent work has revealed that Zfhx3 regulates the expression of Vip and other SCN transcripts, the period of free-running rhythms, and the function of the sleep homeostat (Balzani et al., 2016; Parsons et al., 2015). However, effects of the Zfhx3 mutation do not require its expression during development (Wilcox, Vizor, Parsons, Banks & Nolan, 2017), indicating that Zfhx3 acts to regulate SCN function in adulthood by activating transcription at AT motifs (Parsons et al., 2015). Lastly, the clock genes Rora and Rorb are late-stage markers of SCN development. Rora is expressed in the ventral SCN starting at E14.5, throughout the SCN at E17.5, and in a pattern more restricted to the SCN shell by P21 (Newman et al., 2018; Vandunk et al., 2011). Appropriate expression of Rora/b depends on earlier transcriptional programmes, including Foxd1 and Six3 (Newman et al., 2018; Vandunk et al., 2011). But loss of Rora itself does not disrupt terminal differentiation of VIP or AVP neurons in the SCN (Vandunk et al., 2011), consistent with the later timing of Rora expression relative to other genetic markers. In contrast, a loss-of-function mutation in the Clock gene reduces the postnatal expression of VIP and AVP (Herzog et al., 2000). This is quite interesting given that CLOCK and BMAL are members of the bHLH
class C transcription factor family (Takahashi, 2017), and clock gene expression has been implicated in the control of neurogenesis and differentiation in neurogenic zones of the adult brain (Borgs et al., 2009; Bouchard-Cannon, Mendoza-Viveros, Yuen, Kaern & Cheng, 2013). For instance, adult Per2 and Bmal1 knockout mice display an increased number of newly born and undifferentiated cells in neurogenic zones (Borgs et al., 2009; Bouchard-Cannon et al., 2013). In addition, neurospheres and stem cells cultured in vitro develop molecular rhythms after neuronal differentiation (Malik, Jamasbi, Kondratov & Geusz, 2015; Yagita, et al., 2010), which is abrogated upon cellular reprogramming back into pluripotent cells (Yagita et al., 2010). Furthermore, loss of Clock function adversely affects maturation of neocortical circuits (Kobayashi, Ye & Hensch, 2015). Collectively, this work suggests that the molecular clock can regulate cellular differentiation and circuit formation, but it is also possible that clock genes contribute to this process through non-circadian-related mechanisms. It remains unknown whether the onset of molecular clock function gates SCN development and/or maturation beyond its role in cellular timekeeping.

At E16, SCN progenitors and post-mitotic neuronal precursors display distinct transcriptional profiles (Shimogori et al., 2010). Many genes important for SCN differentiation are maintained after birth (Brown et al., 2017; Vandunk et al., 2011), and expression of Six3, Six6, Lhx1, and Rora fluctuates in a rhythmic manner under either entrained or constant conditions (Clark et al., 2013; Hatori et al., 2014; Vandunk et al., 2011). One of the final stages of terminal differentiation is the adoption of a specific subclass fate, typically defined by neuropeptide expression in SCN neurons (Abrahamson & Moore, 2001; Moore & Speh, 1993; Southey et al., 2014). The timing of GABA and neuropeptide expression will be discussed in a later section because intercellular signalling among SCN neurons is essential for cohesive clock function at the tissue level.

2.4 Process elongation and synaptogenesis

Newly differentiated neurons undergo elongation of axonal and dendritic processes, ultimately culminating in the genesis of synapses (Robichaux & Cowan, 2014; Tretter & Moss, 2008). These two interrelated stages are orchestrated by a variety of mechanisms (e.g., signalling gradients, contact receptors, and cell adhesion molecules), which prompt reorganization of the cytoskeletal proteins actin and tubulin in developing neurons. It has been estimated that the adult rat SCN contains at least $10 \times 10^7$ synapses (Guldner, 1976; Moore & Bernstein, 1989), and that many of these connections arise from neurons located within the nucleus itself (van den Pol & Tsujimoto, 1985; van den Pol, 1980). To our knowledge, this developmental stage has not been examined in the mouse, but there are a handful of anatomical studies investigating synaptogenesis in the rat or hamster SCN (Laemle, Repke, Hawkes & Rice, 1991; Lenn, Beebe & Moore, 1977; Moore & Bernstein, 1989; Speh & Moore, 1993). These studies suggest that there is a progressive increase in the number and complexity of synapses over the early postnatal period, with two intervals of rapid growth corresponding to a large increase in synapse diversity from P0 to P6 and a rapid increase in synapse number from P6 to P10 (Lenn et al., 1977; Moore & Bernstein, 1989).

At E19, SCN neurons in the rat are surrounded by sparse neuropil containing dendritic growth cones and occasional bundles of unmyelinated axons (Moore & Bernstein, 1989). The neuropil and primary dendrites have matured by P6 (Moore & Bernstein, 1989), although further increases in complexity have been noted after P14 (Lenn et al., 1977) possibly due to glial maturation (Munekawa et al., 2000).
From P2 to P6, the diversity of synapses increases, with the appearance of symmetrical, asymmetrical, and intermediate synapses containing clear and/or dense-core vesicles abutting dendritic processes that are variable in size (Lenn et al., 1977). However, at P6, <50% of axon terminals and <30% of synapses are present compared to the adult rat SCN (Moore & Bernstein, 1989). During this early postnatal interval, 80% of dissociated SCN neurons collected from 1- to 3-day-old rats will develop spontaneous inhibitory currents within 4 days in vitro (Welsh, Logothetis, Meister & Reppert, 1995). These electrical responses may be supported either by a low percentage of synapses or could be driven by non-synaptic release of GABA (Demarque et al., 2002). By P10, the complement of axon terminals reaches adult levels and the number of synapses has risen to 70% (Moore & Bernstein, 1989). The remaining synapses may develop in a third wave between P21 and P35 (Lenn et al., 1977). Similarly, synaptogenesis in the hamster SCN occurs from E16 to P4, but has not been investigated as extensively as in the rat (Speh & Moore, 1993).

It remains unclear what mechanisms drive process elongation and synaptogenesis in the developing SCN (Robichaux & Cowan, 2014; Tretter & Moss, 2008). Cell adhesion molecules have been implicated in the process of establishing synaptic connections, but whether these systems direct formation and/or maintenance of SCN circuits remains unclear. In the rat SCN, expression of the cell adhesion molecules NCAM and L1 increases between P7 and P14 (Yamada, Takayama, Seki, Okada & Nagai, 2003). Interestingly, disrupting the function of polysialylated NCAM or L1 in adulthood can impair behavioural rhythms of rats and mice free running under constant conditions (Shen, Watanabe, Tomasiewicz & Glass, 2001; Yamada, Takayama, Okada & Nagai, 1999). Furthermore, loss of NCAM-180 increases the number of VIP neurons in the mouse SCN by 2–3 fold, thus raising the possibility that polysialylated NCAM regulates signalling mechanisms important for SCN function. These studies suggest that cell adhesion molecules regulate SCN development and influence cellular interactions in adulthood either directly or indirectly by changing circuit architecture. In addition, gap junctions have been implicated in the development of chemical synapses in other neural systems (Hormuzdi, Filippov, Mitropoulou, Monyer & Bruzzone, 2004), and SCN cells can be linked by gap junctions (Colwell, 2000; Long, Jutras, Connors & Burwell, 2005; Welsh & Reppert, 1996). Connexin36 is a subunit of gap junctions specifically expressed in neurons, and it has been shown that inhibiting the function of gap junctions eliminates electrical coupling among SCN neurons and deleting connexin36 decreases the amplitude of daily rhythms in behaviour (Long et al., 2005; Wang, Chen & Wang, 2014). However, recent work reveals that loss of connexin36 in adult mice does not impair circadian function at the behavioural or molecular levels (Diemer et al., 2017). Collectively, these results suggest that signalling through connexin36–expression gap junctions modulates short-term electrical coupling among SCN neurons, but this mechanism is not necessary for long-term synchronization among SCN neurons at the level of the molecular clock. Overall, much remains unknown about SCN synaptogenesis, but recent advances in imaging and genetic approaches may provide deeper insight into how these important circuits are constructed and stabilized.

2.5 Neuronal loss
It has been estimated that approximately 50% of neurons produced in the vertebrate brain are lost during development due to a variety of mechanisms (Fricker, Tolkovsky, Borutaite, Coleman & Brown, 2018). The activation of caspase and pro-apoptotic members of the Bcl-2 family is a common cause of cell loss during the post-mitotic phase of development, which overlaps with synaptogenesis.
Both cell-intrinsic programmes and cellular interactions can activate apoptotic pathways during this time. A large number of SCN neurons are lost during the perinatal period, but the mechanisms that activate apoptotic programmes in SCN neurons are unclear. In hamsters, there is a 40% decline in neuronal number over P3–P6 that reduces SCN volume to that typical of adults (Muller & Torrealba, 1998). A similar pattern of developmental cell loss is observed in the mouse SCN (peak P3–P5) using caspase-3 activation (Ahern et al., 2013) or DNA fragmentation as a marker of cell death (Bedont et al., 2014). The timing and patterning of SCN cell death is comparable to other hypothalamic nuclei (e.g., paraventricular nucleus, ventromedial nucleus), suggesting that this is a general process in this region (Ahern et al., 2013). Although the most precipitous cell death occurs in the mouse SCN by P7, an additional 20% cells are lost by adulthood (Bedont et al., 2014). Early genetic programmes influence perinatal cell loss because Lhx1 deletion causes an additional 40% reduction in the number of SCN neurons between P0 and 4 (Bedont et al., 2014). Cell loss in the Lhx1 mutant is preceded by reduced peptide expression, suggesting that cell survival is dependent on terminal differentiation. However, it remains unknown if specific types of SCN neurons are lost during normal cell death and whether the function of the network changes due to their failure to integrate into the circuit.

2.6 Glial development

Throughout the central nervous system, glia are generated after the period of neurogenesis (Rowitch & Kriegstein, 2010). This temporal sequence is thought to reflect that radial glia produce different classes of progenitor pools at early versus late stages of development, with early pools of progenitors producing neurons and later pools producing glia (Tabata, 2015). Earlier-born neural cells influence gliogenesis by releasing extrinsic signals, such as cytokines and Notch ligands (Rowitch & Kriegstein, 2010). Lastly, macroglia can be divided into three distinct types: protoplasmic astrocytes (Aldhl1+, GFAP+ or GFAP−), fibrous astrocytes (AldhL1+, GFAP++), and oligodendrocytes (MBP+, PLP+, APC+), which may be generated from common or independent progenitor pools (Rowitch & Kriegstein, 2010).

Suprachiasmatic nucleus astrocytes have been detected in adult mice, rats, and hamsters using either GFAP or Aldh1L1 expression as markers (Brancaccio, Patton, Chesham, Maywood & Hastings, 2017; Guldner, 1983; Morin, Johnson & Moore, 1989; Tso et al., 2017). Morphological analyses indicate that the rat SCN contains both protoplasmic and fibrous astrocytes that can make appositions onto VIP+ neurons and AVP+ neurons (Tamada et al., 1998). Much like neurons, astrocytes can display daily rhythms in cellular physiology, including those in structural protein expression (Gerics, Szalay & Hajas, 2006; Lindley, Deurveilher, Rusak & Semba, 2008), morphological arrangements (Becquet, Girardet, Guillamond, Francois-Bellan & Bosler, 2008), metabolic function (Burkeen, Womac, Earnest & Zoran, 2011; Womac, Burkeen, Neuendorff, Earnest & Zoran, 2009), and clock gene/protein levels (Cheng et al., 2009; Duhart et al., 2013; Prolo, Takahashi & Herzog, 2005; Tso et al., 2017). Recent work indicates that SCN astrocytes can influence GABA and glutamate signalling in the SCN (Barca-Mayo, et al., 2017; Brancaccio et al., 2017), and that SCN astrocytes affect SCN function in vitro (Brancaccio et al., 2017; Tso et al., 2017). Further supporting a glial role in clock function, modulating the molecular clock specifically in astrocytes will alter the period of circadian rhythms under constant conditions (Barca-Mayo et al., 2017; Tso et al., 2017). Lastly, anatomical and functional evidence suggests that SCN astrocytes may influence photic resetting (Girardet, Becquet, Blanchard, Francois-Bellan & Bosler, 2010; Lavaille & Serviere, 1995; Lavialle, Begue, Papillon & Vilaplana, 2001; Moriya et al., 2000;
Tamada et al., 1998), but deficits in photic responses have not been reported in recent studies genetically manipulating the astrocyte clock.

Although there is a growing appreciation that astrocytes contribute to clock function, the development of SCN glia is not well understood. Most studies have used structural markers to track the development of glia in the SCN. For example, vimentin expression (a marker of radial glia) is high in the SCN prior to birth, but decreases markedly from P3 to P6 in hamsters and rats (Botchkina & Morin, 1995; Munekawa et al., 2000). This likely reflects loss of radial glial progenitors, and its timing is consistent with the postnatal period of cell death in the hamster (Muller & Torrealba, 1998). In both species, there is a postnatal increase in GFAP+ processes that complements the decreasing expression of vimentin. In the hamster SCN, GFAP is first expressed at E15, but large numbers of GFAP+ processes are not detected until P0 (Botchkina & Morin, 1995). From P3 to P10, the number of GFAP+ astrocytes increases further, with expansion continuing into adulthood. Similar development of GFAP expression occurs in the rat SCN, with GFAP expression first evident at E20 and postnatal expansion of GFAP+ processes (Munekawa et al., 2000). Interestingly, this latter study found that GFAP expression appeared in the core and shell SCN at different ages. Specifically, GFAP+ processes first appeared in the ventral SCN and increased from P3 to 4, but GFAP+ processes did not appear in the dorsal SCN until P12. From P20 to P25, GFAP+ increased in both ventral and dorsal SCN, but this process can be affected by changes in light input (Cambras, Lopez, Arias & Diez-Noguera, 2005; Ikeda, Iijima, et al., 2003; Munekawa et al., 2000; Yamazaki, Alones & Menaker, 2002). Collectively, these studies suggest that different pools of SCN astrocytes developing at discrete postnatal stages are sensitive to changes in the perinatal environment. Going forward, deeper insight into SCN macroglia can be gained from fate mapping studies that track astrocyte lineage and transcriptional programming (Rowitch & Kriegstein, 2010; Tabata, 2015). A greater understanding of SCN gliogenesis may allow for more precise ways to manipulate SCN macroglia and their progenitors at critical stages of development.

3 SCN NETWORK DEVELOPMENT

Suprachiasmatic nucleus neurons coordinate their cellular rhythms at the population level (Hastings et al., 2018), and thus the appearance of tissue-level rhythms can be used to infer when SCN circuits have matured. The molecular clock generates circadian rhythms at the cellular level, and intercellular interactions among SCN neurons synchronize, amplify, and stabilize these cellular rhythms (Herzog, Aton, Numano, Sakaki & Tei, 2004). In addition, interactions among SCN neurons generate spatiotemporal gradients in electrical and molecular activity at the network level, which can be adjusted to encode environmental conditions (Evans & Gorman, 2016; Meijer, Michel, Vanderleest & Rohling, 2010). These emergent properties reflect intercellular communication, and numerous chemical signals are produced in the SCN that may modulate network function via synaptic and/or non-synaptic signalling (Castel, Morris & Belenky, 1996; Deery et al., 2009; Maywood, Chesham, O'Brien & Hastings, 2011; Yamaguchi et al., 2003). Specification of neurochemical identity is an important step in terminal differentiation, but postnatal changes in chemical signals are not uncommon. Thus, the role of these chemical signals may vary over development in ways that have important consequences for network maturation (see also Honma review, same issue). In this section, we review the ontogeny of SCN rhythms and the development of the specific chemical signals that are best understood at this time.
3.1 Development of the SCN clock

A long-standing question in the field is when the SCN first starts to function as a clock. Daily fluctuations in metabolic and electrical activity have been detected during late foetal development in rats using a variety of in vivo and acute ex vivo methods. Metabolic rhythms in the rat SCN manifest at E19, with high daytime levels of glucose utilization as in adulthood (Reppert & Schwartz, 1983, 1984; Shibata, Newman & Moore, 1987). Furthermore, SCN slices from foetal rats display higher daytime firing as early as E22 when measured with an ex vivo between-group sampling method (Shibata & Moore, 1987). In this study, daytime firing was low and irregular at E22, but from P1 to P14 gradually adopted more adult-like properties (i.e., increase in regular firing, mean firing rate, and rhythm amplitude). Collectively, these studies reveal that daily rhythms in cellular physiology are expressed prior to birth, which is an age when few synapses are present in the rat SCN (Lenn et al., 1977; Moore & Bernstein, 1989). Furthermore, changes in SCN electrical rhythms after birth indicate that additional maturation of the circuit occurs during the postnatal period of synaptic elaboration and diversification.

Daily rhythms of in vivo clock gene expression also appear during late gestation, although clock genes can become rhythmic at different ages. As mentioned earlier, Rora is first expressed in the mouse at E13.5 and is found throughout the SCN by E17.5 in this species (Vandunk et al., 2011), which is consistent with high constitutive expression of its target Bmal1 at E18 (Ansari, Agathagelidis, Lee, Korf & von Gall, 2009). By E18, Per1 and PER1 are rhythmically expressed in mouse SCN (Ansari et al., 2009; Ohta, Honma, Abe & Honma, 2003; Shimomura, et al., 2001), suggesting that the core molecular loop is functional prior to birth. Studies disagree on whether Per2 and PER2 start to cycle before or after birth (Ansari et al., 2009; Huang, Lu, Chen, Hua & Qian, 2010; Ohta et al., 2003; Shimomura et al., 2001), which may reflect differences in methods or strain/sex of mice used. The last of the core clock components to oscillate appears to be Cry and CRY, with clear daily rhythm s only emerging after P2 (Ansari et al., 2009; Huang et al., 2010). This dovetails with work indicating that Cry genes are not required for SCN rhythms until 1–2 weeks after birth (Ono et al., 2013, 2016; see also Honma review, same issue). Similar staging of molecular rhythms is observed in rats and hamsters, with high early expression of Bmal1 and maturation of molecular rhythms spanning into postnatal life (Houdek & Sumova, 2014; Kovacikova, Sladek, Bendova, Illnerova & Sumova, 2006; Li & Davis, 2005; Sladek et al., 2004). In studies where both positive and negative clock genes are included, it appears that Per1/2 and Bmal1 oscillate out of phase in vivo when they are both rhythmically expressed (Houdek & Sumova, 2014; Kovacikova et al., 2006; Li & Davis, 2005; Sladek et al., 2004). Interestingly, a recent study found that Rev-erba is one of the earliest clock genes to oscillate at E19 in the rat SCN (Houdek & Sumova, 2014). Given that Rev-erba (also known as Nr1d1) negatively regulates Bmal1 expression by competing with Rora (Takahashi, 2017), this suggests that the rhythmic regulation of Bmal1 may be one of the earliest events in the development of the molecular clock. Another consistent pattern across studies and species is that age-related changes in the amplitude and/or phasing of clock gene rhythms manifest in a gene-specific manner, which may reflect further maturation of the molecular clock and/or the influence of external factors (e.g., parturition, light).

Another important question is when the SCN is able to sustain rhythms without maternal influence. This issue has been addressed by examining SCN rhythms over time in culture, which also allows one to study the emergent properties of the network at the cellular and population levels. Pioneering studies employing this approach have demonstrated that SCN neurons collected from P2 to P20 mice can
sustain electrical and molecular rhythms in vitro (Enoki, et al., 2012; Maywood et al., 2006, 2013; Ohta, Mitchell & McMahon, 2006; Yamaguchi et al., 2003). Recent developmental studies using this ex vivo technique have revealed that SCN molecular rhythms are expressed by E15.5 in mice (Carmona-Alcocer et al., 2018; Landgraf, Achten, Dallmann & Oster, 2015; Wreschnig, Dolatshad & Davis, 2014) and by E20 in rats (Nishide, Hashimoto, Nishio, Honma & Honma, 2014). Similar to the development of SCN electrical rhythms, the strength and precision of molecular rhythms continues to increase with age. Real-time imaging of molecular rhythms revealed that only 10% of SCN neurons oscillate at E14.5, but 70% are able to oscillate with high precision by E15.5 (Carmona-Alcocer et al., 2018). Competence of SCN rhythmicity at this age is consistent with work demonstrating that rhythms in SCN-lesioned adults can be restored 90% of time by SCN transplants collected from E14 to 15 foetuses (Kaufman & Menaker, 1993; Romero, Lehman & Silver, 1993). Furthermore, SCN neurons appear capable of forming a cohesive network early in development because period synchrony is evident at E15.5 and the spatiotemporal gradient emerges by P2 (Carmona-Alcocer et al., 2018). Interestingly, phase relationships among different clock genes may also mature late in development because dual real-time reporters indicate the relative phasing of Bmal1 and Per1 rhythms is less stable at P7 compared to adulthood (Ono et al., 2017). Collectively, this work indicates that the maturation of the SCN clock occurs in stages, with early development of oscillator capacity but later development of network properties. Delayed network development is consistent with findings that the SCN is sensitive to perturbation early in development (Landgraf et al., 2015; Nishide, Honma & Honma, 2008; Wreschnig et al., 2014), which may reflect lack of circuit mechanisms that provide robustness (Buhr, Yoo & Takahashi, 2010; Herzog & Huckfeldt, 2003). Furthermore, period synchronization at E15.5 does not require GABA or VIP signalling (Carmona-Alcocer et al., 2018; Wreschnig et al., 2014), indicating that novel signalling mechanisms operate early in development (see also Honma review, same issue).

3.2 Development of specific chemical signals

3.2.1 GABA (γ‐aminobutyric acid)

GABA is the main neurotransmitter produced by SCN neurons (Abrahamson & Moore, 2001; Moore & Speh, 1993), and it is expressed in different peptidergic classes (Castel & Morris, 2000; Francois-Bellan et al., 1990). Synthesis of GABA is controlled by glutamic acid decarboxylase (GAD), and the adult SCN expresses both the GAD65 and GAD67 isoforms (Castel & Morris, 2000; Huhman, Hennessey & Albers, 1996; O’Hara, Andretic, Heller, Carter & Kilduff, 1995). Synaptic release of GABA requires packaging by the vesicular GABA transporter VGAT (encoded by Slc32a1), which is a general marker for GABA neurons (Chen, Wu, Jiang & Zhang, 2017). Lastly, cellular responses to GABA are mediated by GABAA and GABAB receptors. The GABAA receptor is a heteropentameric ligand-gated ion channel permeable to chloride and bicarbonate, with synaptic or extra-synaptic GABAA receptors that differ in subunit composition and functional properties (Albers, Walton, Gamble, McNeill & Hummer, 2017). In the SCN, many GABA responses are driven by GABAA receptor signalling (Jiang, Yang, Liu & Allen, 1997; Liu & Reppert, 2000; Strecker, Wuarin & Dudek, 1997), but the Goi/o-coupled GABAB receptor is also expressed (Belenky, Yarom & Pickard, 2008; Gribkoff, Pieschl & Dudek, 2003).

GABA signalling modulates properties of the SCN network and photic responses in adulthood (reviewed in Albers et al., 2017), but surprisingly little is known about its development in the SCN. During brain development, GABA signalling regulates the formation of neural circuits by influencing cellular proliferation, neuronal differentiation, dendritic outgrowth, synapse formation, and circuit
refinement (Akerman & Cline, 2007). In many developing networks, GABA is the first transmitter to become functional, but this signalling mechanism can operate differently in the perinatal versus adult brain. For instance, GABA can be released as a paracrine signal prior to synaptogenesis, which is facilitated by low transporter activity in perinatal rats and mice (Demarque et al., 2002). In the rat hypothalamus, GABA is expressed in neuronal soma, dendritic growth cones, and growing axons as early as E15 (van den Pol, 1997). Consistent with this embryonic window of GABA expression, Gad1 has been detected in the ventral anterior hypothalamus as early as E16 (Shimogori et al., 2010), and it is likely that the SCN displays postnatal increases in GAD expression similar to that reported for other hypothalamic regions (Popp, Urbach, Witte & Frahm, 2009; Puymirat, Faivre-Bauman, Bizzini & Tixier-Vidal, 1982). Furthermore, Vgat can be detected at low levels in the SCN at E18.5 (Allen Brain Atlas), which is consistent with the timing of Vgat expression in the mediobasal hypothalamus (Kobayashi et al., 2017). After birth, there is a large increase in SCN Vgat expression over the postnatal period of synaptogenesis (Allen Brain Atlas). In general, very little is known about the developmental patterning of GABA receptors in the SCN, however, the postnatal presence of GABA_receptors can be inferred from functional responses to their pharmacological manipulation (Ikeda, Yoshioka & Allen, 2003; Kawahara, Saito & Katsuki, 1993; Welsh et al., 1995). Furthermore, other hypothalamic regions display postnatal swapping of GABA_receptor subunits (Davis, Penschuck, Fritschy, & McCarthy, 2000; Fritschy, Paysan, Enna & Mohler, 1994), but GABA_receptor subunits have been examined in the adult SCN only (Gao, Fritschy & Moore, 1995; O'Hara, Andretic, Heller, Carter, & Kilduff, 1995; Walton, McNeill, Oliver & Albers, 2017).

One interesting feature of GABA responses during development is that there is a postnatal switch from depolarization to hyperpolarization (Ben‐Ari et al., 2012). In the rat hypothalamus, the developmental switch in GABA responses has been estimated to occur from P4 to P10 (Obrietan & van den Pol, 1995), but studies tracking developmental changes in SCN responses to GABA are scarce. In one study, application of GABA was found to increase \([\text{Ca}^{2+}]_i\) in 46% of dissociated SCN neurons collected from E18 rats and cultured for 4 days (Obrietan & van den Pol, 1995). Similarly, application of the GABA_receptor antagonist bicuculline after 6 days in culture depressed \([\text{Ca}^{2+}]_i\) in 50% of these SCN neurons, suggesting that basal \([\text{Ca}^{2+}]_i\) is elevated by GABA released from perinatal SCN neurons. By 18 days in culture, however, GABA increased \([\text{Ca}^{2+}]_i\) in only 13% of SCN neurons. Although dispersed cell cultures may not fully recapitulate the in vivo state, another study has examined \([\text{Ca}^{2+}]_i\) responses in SCN slices collected from mice at discrete postnatal ages (Ikeda, Yoshioka et al. 2003). Overall, this study demonstrates that GABA_receptor activation increases \([\text{Ca}^{2+}]_i\) in 60%–65% of P6–7 SCN neurons, but this is reduced to 26%–40% by P9–P10. Furthermore, the P9–P10 SCN displays a daily rhythm in the \([\text{Ca}^{2+}]_i\) response to GABA_receptor activation that is not present at P6–P7 (Ikeda, Yoshioka et al. 2003). These results suggest that at least a subset of SCN neurons develop phase-dependent responses to GABA_receptor activation, but it remains unknown if this process is influenced by maturation of the molecular clock, cell-type-specific programming, the stabilization of intra-network connections, and/or the arrival of afferent inputs. The factors that regulate this developmental process in the SCN are especially intriguing given that excitatory GABA responses are reported in the adult SCN (reviewed in (Albers et al., 2017) and GABA signalling remains plastic in the adult SCN (Farajnia, van Westering, Meijer & Michel, 2014; Myung et al., 2015). Thus, it is interesting to consider how this developmental process may sculpt GABA responses of adult SCN subclasses and their capacity for plasticity later in life.
3.2.2 Vasoactive intestinal polypeptide

Vasoactive intestinal polypeptide is a member of the secretin family and binds to VPAC1/2 receptors with high affinity (Couvineau & Laburthe, 2011). In the SCN, VIP acts through the VPAC2 receptor, which is expressed by a majority of SCN neurons in the mouse (Kallo et al., 2004; King et al., 2003). When applied to the SCN, VIP and VPAC2 agonists modulate membrane potential, firing rate, \([Ca^{2+}]_i\), and GABA signalling (Irwin & Allen, 2010; Itri & Colwell, 2003; Kudo et al., 2013; Reed et al., 2002). Furthermore, VIP signalling can alter electrical and molecular rhythms in the SCN, which requires activation of PKA, MAPK, and PLC signalling pathways (An, Irwin, Allen, Tsai & Herzog, 2011; Meyer-Spasche & Piggins, 2004; Nielsen, Hannibal & Fahrenkrug, 2002). Loss of VIP signalling will hyperpolarize SCN neurons, decrease the number capable of sustaining circadian rhythms, and compromise synchronization among those that remain oscillating (Aton, Colwell, Harmar, Waschek & Herzog, 2005; Brown, Hughes, & Piggins, 2005; Brown, Colwell, Waschek, & piggins, 2007; Maywood et al., 2006). The importance of VIP is further illustrated by the wide range of circadian phenotypes displayed by mice deficient in VIP signalling, including low-amplitude, short period locomotor rhythms free-running conditions, increased incidence of arrhythmia under constant darkness, accelerated recovery from simulated jetlag, deficits in photoperiodic encoding, and altered responses to constant light (An, et al., 2013; Aton et al., 2005; Bechtold, Brown, Luckman & Piggins, 2008; Colwell et al., 2003; Harmar et al., 2002; Hughes et al., 2015; Loh et al., 2014; Lucassen, et al., 2012). Collectively, these studies indicate that VIP signalling is required to sustain normal SCN function in adulthood and suggest that insight into its functional role may be gained by examining developmental changes in VIP expression.

The onset of Vip expression occurs early in SCN development for many rodent species. In mice, Vip expression occurs by E18.5, which corresponds to 93% GP (Vandunk et al., 2011), which is 3.5 days after the end of neurogenesis in the SCN core in this species (Kabrita & Davis, 2008). This is followed by expression of VPAC2 at P0 and VIP at P2 (Carmona-Alcocer et al., 2018), with further increases in VIP expression between P6 and P30 (Herzog et al., 2000). Rats display similar developmental patterns, with the onset of Vip and VIP expression by E18 (79% GP), increased expression at P0, and two postnatal waves of increased expression between P5–P10 and P10–P20 (Ban, Shigeyoshi & Okamura, 1997; Houdek & Sumova, 2014; Laemle, 1988). In addition, hamsters display VIP by E13–E14 (80%–88% GP) with postnatal increases in expression (Botchkina & Morin, 1995; Romero & Silver, 1990). In all three rodent species, there is postnatal expansion of VIP processes, with a dense plexus formed by P30 in mice (Herzog et al., 2000), by P20 in rats (Ban et al., 1997), and by P10 in hamsters (Botchkina & Morin, 1995). Interestingly, VIP signalling does not appear to be required for SCN synchrony at E15.5 (Carmona-Alcocer et al., 2018; Wreschnig et al., 2014), suggesting that other signalling mechanisms support network function at this age. The loss of VIP signalling reduces the expression of other SCN neuropeptides in adulthood, such as Avp and Prok2 (Bedont et al., 2014; Harmar et al., 2002; Bedont et al., 2018), which could reflect loss of SCN synchrony during adulthood or a developmental deficit in network maturation. With this in mind, it may be interesting to examine whether levels of these other neuropeptides are rescued by treatments that restore intercellular synchrony in VIP-deficient SCN (Aton et al., 2005; Brown, et al., 2007; Hughes et al., 2015; Maywood et al., 2006).
The spatial patterning of postnatal changes in VIP expression has been investigated in both mice and rats, with development in both species characterized by VIP expression expanding into the middle and posterior SCN. In the rat, there are also medial–lateral differences in the timing of Vip expression, with two spatially distinct clusters of VIP neurons that differ in daily VIP expression and molecular responses to light (Ban et al., 1997; Kawamoto et al., 2003). This suggests that there may be two subsets of VIP neurons that differ in the timing of maturation and function within the network. Interestingly, recent work has revealed that VIP neurons in mice can be divided into two subsets based on electrical firing (i.e., tonic versus irregular), and that rapid entrainment can be achieved in vitro or in vivo by driving high-frequency firing from VIP neurons with optogenetic stimulation (Mazuski, et al., 2018).

Collectively, these data suggest the VIP neurons can be divided into at least two subclasses, which may differ in transcriptional programming, cellular physiology, and functional roles.

In addition to the developmental increase in VIP levels, there is an age-related change in the daily rhythm of VIP expression. A daily rhythm in Vip expression is observed in rats as soon as this transcript is detected at E19, but the phasing and amplitude of the rhythm is altered by E21 (Houdek & Sumova, 2014). Changes also occur at postnatal ages, with an inversion of the Vip rhythm under free-running conditions between P10 and P20 (Ban et al., 1997) and a reduction in the amplitude of the VIP rhythm under entrained conditions between P4 and P20 (Isobe & Muramatsu, 1995). During this early postnatal window, VIP can be released rhythmically from organotypic SCN slices collected from P5 to P6 rats (Nakamura, Honma, Shirakawa & Honma, 2001; Shinohara, Honma, Katsuno, Abe & Honma, 1995). But in adult rats, the endogenous rhythm of Vip and VIP expression under constant darkness is lost (Ban et al., 1997; Shinohara, Tominaga, Isobe & Inouye, 1993), suggesting that mechanisms engaged later in life alter the circadian regulation of this peptide. The neurobiological bases and functional implications of these developmental changes remain unknown.

3.2.3 Arginine vasopressin

Recent work indicates that signalling by AVP neurons regulates SCN function in ways beyond its role as an output to downstream tissues (Kalsbeek, Fliers, Hofman, Swaab & Buijs, 2010). The SCN expresses transcripts for the AVP receptors, V1a and V1b (Bedont, et al., 2018), and inhibition of AVP signalling modulates SCN rhythms in vitro by affecting the period and phase relationships of SCN neurons (Bedont et al., 2018; Edwards, Brancaccio, Chesham, Maywood & Hastings, 2016). In vivo, deletion of V1a/b receptors accelerates behavioural and molecular recovery from simulated jetlag, which is mimicked by injections of V1A and V1B antagonists into the SCN (Yamaguchi, et al., 2013). Furthermore, manipulation of the molecular clock specifically in AVP neurons can drive changes in behavioural and SCN rhythms (Mieda et al., 2015; Mieda, Okamoto, & Sakurai, 2016). Evidence indicates that the role of AVP signalling varies over SCN development (Ono, Honma & Honma, 2016), suggesting that age-related changes in AVP expression influence maturation of the master clock network.

Suprachiasmatic nucleus expression of Avp occurs at a later embryonic age relative to other hypothalamic nuclei. In the mouse, Avp is first detected in the SON at E14, in the PVN at E16, and in the SCN at E18 (Hyodo, Yamada, Takezawa & Urano, 1992; Jing, Ratty & Murphy, 1998; Vandunk et al., 2011). The staging of Avp expression across these three structures corresponds with regional differences in the timing of neurogenesis for AVP neurons (Okamura et al., 1983). In addition, the
onset of \textit{Avp} expression in the SCN may be gated by maturation of the molecular clock as this transcript is a first-order clock-controlled gene (Jin et al., 1999). In the mouse SCN, AVP expression is evident by P3–P6, and there is a large postnatal increase in AVP production where adult levels are achieved by P20 (Herzog et al., 2000; Hyodo et al., 1992). Similar developmental patterns of \textit{Avp} and AVP expression have been reported in both rats and hamsters (de Vries, Buijs & Swaab, 1981; Delville, Mansour, Quan, Yules & Ferris, 1994; Houdek & Sumova, 2014; Reppert & Uhl, 1987). In rats, \textit{Avp} is expressed rhythmically as soon as the transcript is detected (Houdek & Sumova, 2014; Reppert & Uhl, 1987), but there are subsequent changes in the time-of-peak transcription from E19 to E21 (Houdek & Sumova, 2014) and peak protein expression from P0 to P20 (Isobe & Muramatsu, 1995).

Comparison of AVP and VIP specificat\textit{ion may speak to whether different peptidergic classes of SCN neurons are similar in the staging of critical developmental events. Transcripts of both peptides are expressed by E19 in the mouse and rat SCN (Houdek & Sumova, 2014; Vandunk et al., 2011). In the mouse SCN, the \textit{Avp} transcript appears approximately 2 days after the end of neurogenesis in AVP neurons (Okamura et al., 1983), whereas \textit{Vip} expression occurs within 3–4 days after the end of neurogenesis in the SCN core (Kabrita & Davis, 2008; Vandunk et al., 2011). This suggests that there may be a longer interval to VIP production, but the precise time of neurogenesis in VIP neurons has only been identified in the hamster (Antle, LeSauter 2005). Similarly, the two transcripts differ in how their rhythms change with age. Rhythmic expression of both transcripts in the rat SCN occurs within 1–2 days after detection, but the phasing and amplitude for both is altered over subsequent days (Houdek & Sumova, 2014). Interestingly, both the \textit{Avp} and \textit{Vip} rhythms shift by approximately 6 hr over E19–E21, but \textit{Avp} amplitude increases and \textit{Vip} amplitude decreases. Both peptides appear early in postnatal development, with detectable levels of VIP at P2 (Carmona-Alcocer et al., 2018) and AVP by P3–P6 (Herzog et al., 2000). Over the subsequent 1–3 weeks, both proteins display a marked increase in expression together with changes in the phasing and/or amplitude of daily expression patterns. These perinatal changes in transcript/protein could occur at the single-cell level or could be influenced by new sets of neurons that have expression later in life. Detailed investigations comparing the timing of specification and maturation of different neuronal subclasses in the same species may provide insight into potential factors regulating developmental staging in different types of SCN neurons.

3.2.4 Other signalling peptides

Two additional neuropeptides expressed in the SCN core are gastrin-releasing peptide (GRP) and Substance P, both of which are involved in photic responses. GRP is produced in mice, rats, and hamsters (Guillaumond et al., 2007; Karatsoreos, Romeo, McEwen & Silver, 2006; LeSauter, Kriegsfeld, Hon & Silver, 2002), but Substance P expression is more variable across rodent species (LeSauter et al., 2002; Shibata, Tsuneyoshi, Hamada, Tominaga & Watanabe, 1992; Silver et al., 1999). Furthermore, the spatial location of receptors for each neuropeptide appears different, with the GRP receptor (the \textit{Gq}−coupled Bombesin 2 receptor, BB2) most densely expressed in the SCN shell (Aida et al., 2002), and the Substance P receptor (NK-1) largely confined to the SCN core (Piggins, Samuels, Coogan & Cutler, 2001). Likewise, there is functional evidence that these two neuropeptides have different roles in photic processing. Exogenous application of either GRP or Substance P can mimic the resetting actions of light, but only GRP elicits light-like responses during both early and late subjective night (Aida et al., 2002; Albers, Gillespie, Babagbemi & Huhman, 1995; Antle, Kriegsfeld & Silver, 2005; Gamble, Allen, Zhou & McMahon, 2007; Kallingal & Mintz, 2014; LeSauter et al., 2002; McArthur, et
VIP, GRP, and Substance P are all SCN core peptides that contribute to photic processing, but there are differences in their developmental patterning. In the hamster, VIP, GRP, and Substance P neurons are generated at a similar embryonic age (Antle, LeSauter 2005), but the timing of neuropeptide expression differs (Antle, LeSauter, 2005; Botchkina & Morin, 1995; Romero & Silver, 1990). Expression of GRP and Substance P occurs between P8 and P10 in the hamster SCN, whereas VIP is produced much earlier at E13–E14 (as discussed in above section). Likewise, a developmental delay between VIP and GRP expression occurs in the mouse SCN (Carmona-Alcocer et al., 2018; Drouyer, LeSauter, Hernández & Silver, 2010) and the rat SCN (Ban et al., 1997; Isobe & Muramatsu, 1995; Takatsuji, Miguel-Hidalgo, & Tohyama, 1991). The stark difference in the timing of VIP and GRP is surprising given their parallels in the adult SCN. Like VIP neurons, GRP neurons in the mature SCN are retino-recipient (Drouyer et al., 2010; Fernandez, Chang, Hattar & Chen, 2016; Karatsoreos, Yan, LeSauter & Silver, 2004; Lokshin, LeSauter & Silver, 2015; Tanaka et al., 1997) and display light-induced increases in firing rate, immediate early gene expression, and neuropeptide release (Earnest, DiGiorgio & Olschowka, 1993; Francl et al., 2010; Gamble, Kudo, Colwell & McMahon, 2011; Guillaumond et al., 2007; Lesauter, Silver, Cloues & Witkovsky, 2011; Romijn, Sluiter, Pool, Wortel & Buijs, 1996). In addition, potential interactions between GRP and VIP are illustrated by their co-expression in a subset of SCN neurons (Okamura et al., 1986; Romijn, Sluiter, Wortel, Van Uum & Buijs, 1998) and synergistic responses to GRP and VIP co-administration (Albers Liou, Stopa, & Zoeller, 1991; Albers et al., 1995; Chan et al., 2016). Furthermore, BB2 signalling can synchronize SCN neurons in the absence of VIP signalling (Brown et al., 2005; Maywood et al., 2011). But unlike VIP, there is no evidence that GRP is required for SCN timekeeping because BB2-deficient mice exhibit deficits in photic responses but otherwise normal rhythms in behaviour and SCN function (Aida et al., 2002). Thus, it is tempting to speculate that the later emergence of GRP expression during development is related to its more specific role in adult SCN function.

3.2.5 Other cellular markers
A subset of SCN neurons expresses the calcium-binding proteins, Calbindin and Calretinin, which are intracellular calcium buffers that play a role in calcium sensing and/or transport (Schmidt, 2012; Schwaller, 2014). Calbindin can be used as a cell-type marker, although this buffer is often co-expressed with other core peptides such as VIP, GRP, and Substance P (Drouyer et al., 2010; Lesauter et al., 2002). In adulthood, expression of each calcium buffer can fluctuate over the 24 hr day, with higher Calbindin expression during the night (Arvanitogiannis, Robinson, Beaule & Amir, 2000; LeSauter, Bhuiyan, Shimazoe & Silver, 2009) and higher Calretinin expression during the day (Arvanitogiannis et al., 2000; Moore, 2016). In the adult SCN, the expression of Calbindin and Calretinin varies among rodent species. The hamster displays both Calbindin and Calretinin in the SCN core, with dense expression of Calbindin in a compact subnucleus (Antle, Kriegsfeld 2005; Antle, LeSauter 2005; Marshall, Fa’anunu & Bult, 2000; Silver et al., 1996). In the adult rat, Calbindin is expressed in the SCN core as well (Arvanitogiannis et al., 2000), but Calretinin is expressed in the SCN shell (Marshall et al., 2000; Piggins, Goguen & Rusak, 2005; Shibata et al., 1992; Sterniczuk, Colijn, Nunez & Antle, 2010). In addition, GRP is rhythmically expressed in a species- and/or age-dependent manner (Francl, Kaur & Glass, 2010; Isobe & Muramatsu, 1995; Karatsoreos et al., 2006; McArthur et al., 2000; Okamura & Ibata, 1994; Shinohara et al., 1993), but levels of Substance P do not appear to fluctuate (Otori et al., 1993).
Lastly, Calbindin is diffusely expressed in the adult mouse SCN, and Calretinin is more concentrated in the SCN core (Silver et al., 1999). Despite species differences in Calbindin expression in adulthood, work conducted to date suggests an important role for this calcium buffer in daily timekeeping in both hamsters and mice. In the hamster, Calbindin-expressing SCN neurons influence daily timekeeping and photic responses (Antle et al., 2003; Hamada et al., 2003; LeSauter & Silver, 1999), and microlesions targeting this specific SCN subpopulation has been shown to eliminate overt rhythms (Kriegsfeld, LeSauter & Silver, 2004). Similarly, Calbindin−/− mice exhibit deficits in photic entrainment together with low-amplitude rhythms or arrhythmia under constant darkness (Kriegsfeld et al., 2008), which suggests a role for this calcium buffer in regulating the function of the developing and/or mature SCN.

To our knowledge, development of calcium buffers has been examined only in postnatal hamsters and mice. In the hamster SCN, Calbindin-expressing neurons are generated during a similar embryonic window as other SCN core neurons (Antle, LeSauter 2005), and Calbindin is present at birth with postnatal increases in expression until adult levels are achieved at P15 (Antle, LeSauter 2005). The developing mouse SCN also displays postnatal changes in Calbindin expression, but levels decrease with age in this species and the spatial pattern becomes more diffuse (Ikeda & Allen, 2003; Kriegsfeld et al., 2008). From P3 to P16, high levels of Calbindin are localized to a central subregion of the mouse SCN core, but this region-specific pattern is lost by adulthood (Ikeda & Allen, 2003; Kriegsfeld et al., 2008; Silver et al., 1999). Furthermore, it has been reported that Calretinin levels increase from P9 to P20 (Ikeda & Allen, 2003), suggesting a developmental switch in calcium buffers in the SCN that is opposite to that observed in newborn granule cells of the hippocampus (Todkar, Scotti & Schwaller, 2012). It has been proposed that Calbindin and Calretinin protect neurons against excitotoxicity and apoptotic cell death due to their capacity for “fast” calcium-buffering properties (Choi, Lee, Lim & Oh, 2008; D’Orlando et al., 2001; D’Orlando, Xelio, & Schwaller, 2002). In this light, it is interesting to note that the developmental period of high Calbindin expression in the mouse SCN overlaps with the interval of depolarizing responses to GABA (Ikeda, Yoshioka et al., 2003; Obrietan & van den Pol, 1995), maturation of retinal inputs (McNeill et al., 2011; Sekaran et al., 2005), and cell loss in this species (Ahern et al., 2013; Bedont et al., 2014). Of note, Calbindin−/− mice display a normal complement of VIP neurons, but a decrease in the number of AVP-immunoreactive neurons (Kriegsfeld et al., 2008). Circadian deficits in Calbindin−/− mice relate to the loss of this calcium buffer during a critical period of SCN development (Kriegsfeld et al., 2008), but the precise mechanisms remain unclear.

4 CONCLUSIONS
The development of the SCN network is a gradual process that spans both embryonic and postnatal ages (Figure 1d). Recent work using real-time reporters of molecular rhythms dovetails with classic studies to support the idea that SCN neurons display the capacity for circadian timekeeping soon after the close of neurogenesis. Nevertheless, postnatal maturation of SCN circuits occurs at both the molecular and network levels. At the molecular level, this is best exemplified by evidence indicating that clock genes are expressed and become rhythmic at different developmental stages. At the network level, postnatal maturation of the SCN includes the progressive introduction, elaboration, and rewiring of signalling mechanisms that support master clock function in adulthood. In many respects,
the precise mechanisms guiding the development of SCN cells and circuits are not well understood, but the current gaps in understanding provide promising avenues for future research.

Genetic programming and intercellular signalling guide SCN development, but there is still much to learn about the relative contribution and interplay of these two factors. For instance, to what extent do intrinsic genetic programmes and intercellular signalling interact to guide induction and refinement of the SCN network? Do SCN cells communicate with one another to influence their mutual development? For example, SCN neurons that mature early in development might produce signals (e.g., GABA, VIP) that could set the stage for the later specification of other cells in a homo- or heterotypic manner. In other regions of the hypothalamus, neuropeptides can influence proliferation, differentiation, and synaptic wiring (Bakos, Zatkova, Bacova & Ostatnikova, 2016). Thus, it is possible that SCN neuropeptides play a similar role during the development of master clock circuits. In addition, it would be interesting to examine what cues and factors regulate neurite elongation, synaptogenesis, cell survival, and neuronal loss in the developing SCN. This may provide new insights into whether SCN circuits are rearranged and modified after they are initially established. Recent advances in genetic labelling of specific pools of post-mitotic neurons may aid in pursuing the answers to some of these questions.

Another issue that warrants further examination is whether the timing of key developmental checkpoints is generic to all SCN neurons or whether there are cell-type–specific programmes at play. Some support for the latter model derives from evidence that the onset of neuropeptide expression can differ markedly among classes of SCN neurons that appear to be generated during a similar embryonic window. For example, neurons that will produce VIP, GRP, Substance P, and Calbindin are born at a similar embryonic age in the hamster SCN, but the onset of neuropeptide expression appears to vary substantially among these subclasses. Although it is difficult to precisely pinpoint the exact age at which peptides are produced due to developmental changes in levels or rhythms of expression, it is tempting to speculate based on this evidence that the timing of differentiation and specification varies among distinct classes of SCN neurons. Might there be other phenotypic checkpoints that differ among SCN subclasses, such as the maturation of molecular clock function, neurite elongation, or receptor expression? If subclasses of SCN neurons do mature in a cell-type–specific manner, it would be of interest to determine what extent these programmes are guided by cell-intrinsic and/or cell-extrinsic factors.

Given evidence that perinatal photic conditions can influence circadian function in both humans and animal models, a key area for future research is to investigate the basis of this effect. Although this review has focused on developmental events within the SCN itself, there is mounting evidence that maturation of the SCN network is influenced by signals from the eye. Light is transmitted to the SCN by the retinohypothalamic tract (RHT), which is formed by axons from intrinsically photosensitive retinal ganglion cells (ipRGCs) that express the photopigment melanopsin and relay signals from the rod and cone photoreceptors (Van Gelder & Buhr, 2016). Neurogenesis of ipRGCs occurs during E11–E19 in the mouse (McNeill et al., 2011), with the expression of melanopsin by E11–E15 in the mouse retina (McNeill et al., 2011; Tarttelin et al., 2003) and by E18 in the rat retina (Fahrenkrug, Nielsen & Hannibal, 2004). The first RHT terminals innervate the rat and mouse SCN by P0 (McNeill et al., 2011; Speh & Moore, 1993), and by this age ipRGCs in the mouse are capable of mounting light-evoked
electrical responses that can increase immediate early gene expression in the SCN (Hannibal & Fahrenkrug, 2004; Sekaran et al., 2005). Despite early RHT innervation by a small number of terminals, there is a prolonged period of postnatal expansion and elaboration over the first 2 weeks after birth that corresponds to greater SCN responses to light (Kaufman & Menaker, 1994; Lupi, Sekaran, Jones, Hankins & Foster, 2006; Mateju et al., 2009; McNeill et al., 2011; Munoz Llamosas, Huerta, Cernuda-Cernuda & Garcia-Fernandez, 2000; Speh & Moore, 1993). This postnatal window coincides with several critical events in the SCN, including cell loss, synaptogenesis, glial maturation, the switch in GABA responses, and changes in neuropeptide expression. Evidence that the RHT influences SCN development is provided by work demonstrating that enucleation and/or dark rearing will decrease VIP, increase AVP, decrease Substance P, and modulate GFAP expression (Ban et al., 1997; Cambras et al., 2005; Ikeda, Iijima, et al., 2003; Isobe & Muramatsu, 1995; Munekawa et al., 2000; Smith & Canal, 2009; Takatsuji et al., 1991). This is matched by work indicating that the perinatal lighting environment can influence SCN function and behavioural rhythms later in life (Cambras, Canal, Torres, Vilaplana & Diez-Noguera, 1997; Chew et al., 2017; Ciarleglio, Axley, Strauss, Gamble & McMahon, 2011), including modulation of key properties such as free-running period length. The neurobiological basis of these effects remains unclear, but may relate directly to changes in neuropeptide expression as these signals serve as both important modulators of the SCN network and as daily outputs to downstream clock tissues. The precise mechanisms by which the retina influences the composition and function of the SCN network are important areas for future research.

Several principles of circadian development gleaned from rodent studies appear to generalize to humans and non-human primates. The emergence of foetal rhythms during embryonic development is common in many mammalian species (Mirmiran & Ariagno, 2000; Seron-Ferre, Valenzuela & Torres-Farfan, 2007). In humans and nonhuman primates, daily rhythms in foetal heart rate, respiration, hormone production, and body movement can be detected during late gestation. Daily rhythms in body temperature can be detected in 50% of babies born prematurely (Mirmiran & Ariagno, 2000), but body temperature rhythms are low amplitude in most newborns (Glotzbach, Edgar & Ariagno, 1995). Over the ensuing months, daily rhythms re-emerge in the neonate with distinct rhythms emerging at different ages (Glotzbach et al., 1995; Kennaway et al., 1996; Kleitman & Engelmann, 1953; McGraw et al., 1999; Price et al., 1983; Rivkees, 2007). The prenatal emergence of overt rhythms in primates appears to coincide with the onset of molecular clock function in the SCN, although there are very few studies examining this issue. In the capuchin monkey, Bmal1, Per2, Cry2, and Clock are expressed by an age corresponding to 90% gestation (Torres-Farfan et al., 2006). Daily fluctuations in Bmal1 and Per2 are evident at this age, although the phasing and amplitude of these rhythms are not identical to adults. Lastly, sequential expression of VIP and AVP occurs in humans prior to birth, with postnatal increases in the number of SCN neurons until 1–3 years of age (Swaab, Van Someren, Zhou & Hofman, 1996; Xu, Hu, Wu & Zhou, 2003). Collectively, these data suggest that SCN development in both primates and rodents is characterized by an extended interval of postnatal maturation at the molecular and network levels. Against this backdrop, many investigators have warned about the potential harm caused by circadian disruption during perinatal development (Amaral et al., 2014; Mirmiran & Ariagno, 2000). There is an increasing appreciation of the health costs of circadian disruption in adulthood (Evans & Davidson, 2013), and circadian disruption during gestation may have detrimental consequences for both mother and child (Amaral et al., 2014). Additional work
aimed at decoding the mechanisms that guide SCN development is expected to provide a solid foundation upon which to better understand the sources and effects of circadian disruption early in life. To achieve this goal, it will be important to study the precise mechanisms that guide SCN development in both rodent and non-human primates, as well as the effects of circadian disruption on short- and long-term health outcomes.

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CONFLICT OF INTEREST
The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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