Molecules and Cells

Minireview

Ultradian Rhythms in the Hypothalamic Arcuate Nucleus Kisspeptin Neurons and Developmental Processes

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Numerous physiological processes in nature have multiple oscillations within 24 h, that is, ultradian rhythms. Compared to the circadian rhythm, which has a period of approximately one day, these short oscillations range from seconds to hours, and the mechanisms underlying ultradian rhythms remain largely unknown. This review aims to explore and emphasize the implications of ultradian rhythms and their underlying regulations. Reproduction and developmental processes show ultradian rhythms, and these physiological systems can be regulated by short biological rhythms. Specifically, we recently uncovered synchronized calcium oscillations in the organotypic culture of hypothalamic arcuate nucleus (ARN) kisspeptin neurons that regulate reproduction. Synchronized calcium oscillations were dependent on voltage-gated ion channel-mediated action potentials and were repressed by chemogenetic inhibition, suggesting that the network within the ARN and between the kisspeptin population mediates the oscillation. This minireview describes that ultradian rhythms are a general theme that underlies biological features, with special reference to calcium oscillations in the hypothalamic ARN from a developmental perspective. We expect that more attention to these oscillations might provide insight into physiological or developmental mechanisms, since many oscillatory features in nature still remain to be explored.

Keywords: calcium oscillation, development, kisspeptin, reproduction, ultradian rhythm

INTRODUCTION

Numerous processes in nature possess rhythmic and repetitive oscillations within a specific time period, ranging from calcium oscillations in cells and the sleep-wake cycle to the reproductive cycle (Goldbeter, 2008). Biological rhythms include the circadian rhythm that has a period of approximately 24 h, the infradian rhythm that has a period longer than 24 h, and the ultradian rhythm that occurs multiple times within 24 h (Dibner et al., 2010; McGinnis and Young, 2016). While biological rhythms can be generated by self-sustained oscillators that are maintained in the absence of environmental cues, external signals such as photoperiod or hormonal signals can entrain the rhythm (Gachon et al., 2004).

The most characterized biological rhythms so far are circadian rhythms. The 2017 Nobel Prize in Physiology or Medicine was awarded to three researchers for the discovery of molecular mechanisms controlling the circadian rhythm. The molecular circadian clock is composed of a transcriptional-translational feedback loop of activators and repressors that can control the rhythmicity of their own expression (Takahashi, 2017). In mammals, the suprachiasmatic nucleus (SCN) in the hypothalamus has been identified as the master

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circadian pacemaker (Hastings et al., 2018; Takahashi, 2017). The molecular machinery of the circadian clock is present in many plant, animal, fungal, and cyanobacterial species, and is similar in principle (Edgar et al., 2012).

In contrast to the circadian rhythm, the central pacemaker and molecular mechanisms of ultradian rhythms have not yet been elucidated. One of the well-known examples of an ultradian rhythm includes the hypothalamic gonadotropin-releasing hormone (GnRH) pulse generator that governs pituitary secretion of gonadotropins, which is important for the regulation of mammalian reproduction (Herbison, 2018; McCartney and Marshall, 2014). Kisspeptin neurons in the hypothalamic arcuate nucleus (ARN) appear to be essential components that control GnRH neurons in the initiation and maintenance of development and reproduction, but the origin of the pulse generator remains elusive. Here, we briefly review the ultradian rhythm with a special reference to the calcium oscillations in the hypothalamic ARN and from a developmental perspective.

ULTRADIAN RHYTHMS IN PHYSIOLOGICAL PROCESSES

Ultradian rhythms maintain the stability of living organisms by preparing the system to respond and adapt quickly to external signals. These oscillations can be considered as homeodynamic conditions that involve constantly changing self-organization processes (Yates and Yates, 2008). Cellular processes such as energy production, biosynthetic pathways, protein assembly, stress response, and cell differentiation/division depend on the coordination of ultradian organization (Cortassa et al., 2011). Homeodynamic conditions allow living systems to coordinate their function in a spatio-temporal manner and organize the complexities of life (Lloyd et al., 2001). Loss of ultradian rhythmicity can lead to dysregulation of physiological processes. One long-studied example is related to reproduction. In hypothalamic-lesioned monkeys, intermittent delivery of GnRH established pulsatile gonadotropin secretion, while constant infusion failed to restore gonadotropin secretion (Belchetz et al., 1978). Discovery of the mode of GnRH secretion has been utilized in therapeutics for infertility such as hypogonadotropic hypogonadism.

Another prominent example is the blood level of glucocorticoid (GC). Often, ultradian rhythms are related to circadian rhythms. GC is an adrenal steroid secreted from the adrenal gland, which has a peripheral clock and generates daily oscillations of GC (Son et al., 2008). Interestingly, in the pattern of GC secretion, an hourly ultradian pulsatility exists together with a circadian rhythm to maintain stress responsivity and to prevent the downregulation of GC signaling systems (Lightman and Conway-Campbell, 2010). Nonpulsatile GC rhythm changes the neural processing of emotional input and further regulates cognitive or behavioral outcomes (Kalafatakis et al., 2018).

There are many other forms of ultradian rhythms in diverse physiological functions. Sleep, a representative circadian phenomenon, is also composed of an ultradian rhythm of rapid eye movement (REM)/non-REM (NREM) sleep cycles that have a period of about 90 min (Lamont and Amir, 2010). Ultradian sleep cycles are established during early childhood, and the duration of the ultradian REM/NREM cycle increases with age to a more adult-like pattern (Lopp et al., 2017). In a special case, oxytocin neurons in the hypothalamus display synchronized bursting electrical activity during lactation, every 3 to 15 min to induce milk ejection (Israel et al., 2003). As another example, ultradian locomotor rhythms in voles rely on the dopamine ultradian oscillator (DUO), which cycles in harmony with the circadian clock, and secreted dopamine levels fluctuate in synchrony with ultradian activity cycles (Blum et al., 2014; Bourguignon and Storch, 2017). Furthermore, calcium oscillations in cells can represent signals to transmit biological information that can be modulated by frequency or amplitude (Smedler and Uhlen, 2014). Synchronized calcium signals in hypothalamic slices of the subparaventricular zone and paraventricular nucleus (SPZ-PVN) showed an ultradian rhythm that was superimposed on the circadian rhythm in the SCN (Wu et al., 2018).

CALCIUM OSCILLATIONS IN HYPOTHALAMIC ARN KISSPEPTIN NEURONS AND INTERACTIONS WITHIN THE ARN

To elucidate the origin of the GnRH pulse generator, mathematical modeling and experimental confirmation were performed to modulate luteinizing hormone secretion by activating kisspeptin neurons (Voliotis et al., 2019). Thus, methods to measure ultradian rhythms exist, and research to find the mechanisms underlying ultradian rhythms is ongoing. Meanwhile, the hypothalamic ARN consists of a heterogeneous neural population including kisspeptin neurons. Kisspeptin neurons are critical regulators of mammalian reproduction. Mutations in the kisspeptin receptor caused hypogonadotropic hypogonadism in humans and mice, suggesting that its signaling is essential for puberty (Seminara et al., 2003). Consequential studies supported that kisspeptin neurons are upstream regulators of GnRH neurons and the hypothalamic-pituitary-gonadal axis that govern reproductive maturation (Pinilla et al., 2012). Kisspeptin neurons are immunohistochemically detected from embryonic day 16.5, and by that time they are synaptically connected to GnRH neurons (Kumar et al., 2015), unlike another kisspeptin population in the anteroventral periventricular nucleus (AVPV), which is detected at later developmental stages (Clarkson and Herbison, 2006). Previously, our group reported that intermittent administration of kisspeptin activated pulsatile GnRH transcription and secretion (Choe et al., 2013). GnRH secretion is observed soon after birth in the neonatal period (sometimes called the minipuberty of early infancy), then, after a quiescent juvenile period, reactivated from the peripubertal period (Fig. 1A), and the activity of GnRH is thought to involve the input from kisspeptin neurons (Herbison, 2016). During the follow-up study, we examined whether ARN kisspeptin neurons can generate pulsatility in the organotypic culture of neonatal mice. Kisspeptin neuron-specific calcium imaging from organotypic slice cultures revealed that ARN kisspeptin neurons generate self-sustained and synchronized calcium oscillations with an approximately 3-min period *ex vivo* (Kim et al., 2020).

In order to explore the factors that are involved in regu-

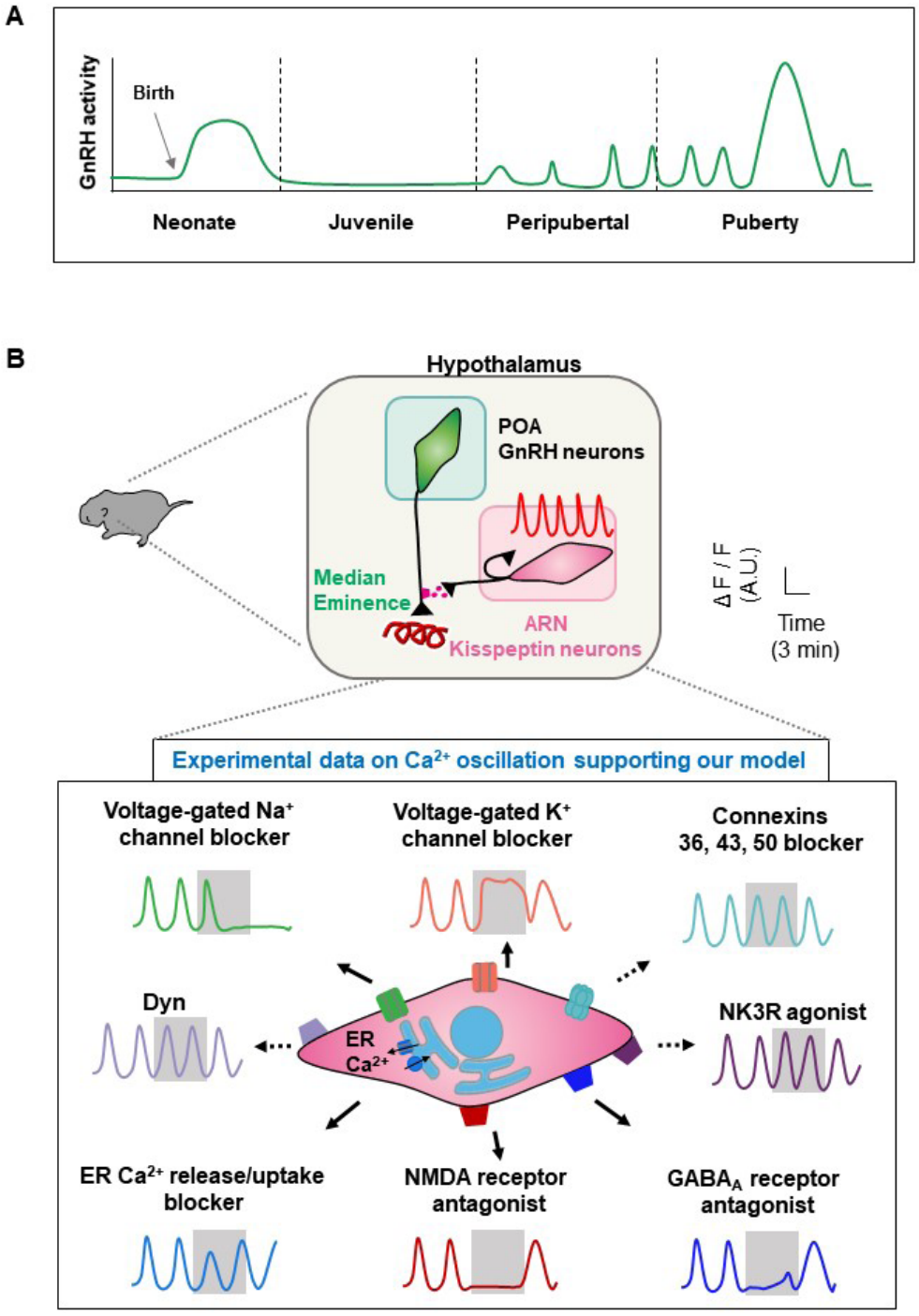

Fig. 1. GnRH neuron activity and regulatory factors of ARN kisspeptin neuron-specific ultradian calcium oscillations in neonates. (A) After birth, GnRH neurons in neonates show an early increase in activity. After suppression of the activity during the juvenile period, the GnRH pulse is reactivated in the peripubertal period. In females, the late development of the AVPV kisspeptin neurons in the pubertal period enables generation of the GnRH surge (Herbison, 2016). (B) In neonatal mice, hypothalamic ARN kisspeptin neurons are connected to GnRH neurons that have cell bodies in the preoptic area (POA) and axon terminals in the median eminence. ARN kisspeptin neuronal networks can generate ultradian calcium oscillations that are dependent on voltage-gated sodium (Na^+) or potassium (K^+) channels and endoplasmic reticulum (ER)-dependent calcium (Ca^{2+}) release or uptake. However, signaling through neurokinin B receptor (NK3R) or dynorphin (Dyn), which are known to auto-regulate ARN kisspeptin neurons, or gap junctions composed of connexins 36, 50, or 43, were marginally effective at the neonatal stage. Furthermore, N-methyl-D-aspartate (NMDA) receptor- and gamma-aminobutyric acid (GABA) type A receptor-mediated neurotransmission were involved in regulating the ultradian calcium oscillations.

lating these unique calcium oscillations, various possibilities were pharmacologically investigated (Fig. 1B). Voltage-gated sodium or potassium channel blockers disturbed calcium oscillations, indicating that action potentials are critical for generating oscillations. Chemogenetic inhibition using designer receptors exclusively activated by designer drug (DREADD) systems suggested that network interactions within ARN kisspeptin neurons are involved in mediating synchronized oscillations. Notably, signaling through neurokinin B (NKB) or dynorphin (Dyn), which is known to auto-regulate ARN kisspeptin neurons (also called KNDy neurons), was marginally effective at the neonatal stage. Furthermore, synchronized calcium oscillations were dependent on intracellular calcium regulation in the endoplasmic reticulum, but were not regulated by gap junctions composed of connexins 36, 50, or 43. Finally, N-methyl-D-aspartate (NMDA) receptor- and gamma-aminobutyric acid (GABA) type A receptor-mediated neurotransmission were involved in regulating ultradian calcium oscillations (Kim et al., 2020).

In organotypic slice cultures, kisspeptin neurons maintained their calcium rhythmicity depending on the network interaction within the ARN. Analysis of hypothalamic ARN and median eminence identified distinct clusters of neural cells (Campbell et al., 2017). The ARN is composed of heterogenous populations as shown in the clustering results of single cell RNA-seq (Fig. 2A). Among the populations, the satiety and hunger sensing neurons proopiomelanocortin (POMC) and agouti-related peptide (AgRP) which play opposite roles in energy homeostasis, are present that can be regulated by kisspeptin, suggesting a functional relationship between energy homeostasis and reproduction within the ARN (Fu and van den Pol, 2010). Ghrelin, a 'hunger' hormone released from the gastrointestinal tract, shows ultradian fluctuations (Yildiz et al., 2004) and increases the activity of AgRP/NPY while inhibiting that of POMC (Muller et al., 2015). Another population, dopaminergic neurons that inhibit prolactin release, are also regulated by kisspeptin (Ribeiro et al., 2015; Szawka et al., 2010). In particular, as mentioned in the previous section, DUO operates in the mammalian brain (Blum et al., 2014; Bourguignon and Storch, 2017). Collectively, these lines of evidence suggest that neuronal subtypes of ARN may coordinate kisspeptin neurons' calcium oscillations through local neuronal interactions. We believe that future studies on these neuronal networks need to consider the heterogeneity of the ARN population.

ARN kisspeptin neurons can generate ultradian calcium oscillation early on, from the postnatal stage, which can possibly reshape with development. Fiber photometry of ARN kisspeptin neurons in adult mice exhibited calcium oscillations with a period of approximately 9 min (Clarkson et al., 2017), which is longer than the oscillation in neonates *ex vivo* (3 min). Between neonate and adult mice, numerous developmental changes take place (Fig. 2B). For example, the expression levels of the neuropeptides' receptors implicated in the auto-regulation of KNDy neurons are low in neonates (Kim et al., 2020) and increase with developmental maturation (Gill et al., 2012; Navarro et al., 2012). The physiological significance of these short calcium oscillations remains to be elucidated.

ULTRADIAN OSCILLATIONS IN DEVELOPMENTAL PROCESSES

Ultradian rhythms are also present in many phenomena during developmental processes. The basic helix-loop-helix transcription factors Achaete-scute homolog (Ascl1) and Hairy and enhancer of split-1 (Hes1), which regulate cell proliferation and differentiation, are expressed in an oscillatory manner to maintain proliferating neural progenitor cells, while sustained expression promotes neuronal fate determination (Imayoshi et al., 2013). Hes1 transcription is repressed by Hes6, a transcriptional regulator that shows rhythmic expression mediated by sumoylation (Lee et al., 2015). Additionally, Wnt and Notch signaling oscillations are functionally linked during segmentation of mouse embryonic mesoderm, and anti-phase oscillations impair physical segment formation (Sonnen et al., 2018).

In the developing embryo, the presomitic mesoderm is segmented into somites in a periodic manner by rhythmic gene expression along the antero-posterior axis (Kageyama et al., 2009). The periodic formation of somites is evolutionarily conserved across several vertebrate species (Wolpert et al., 2015): 90 min in chick, 120 min in mouse, 45 min in Xenopus, 30 min in zebrafish, and 6 h in humans. These oscillations in the ultradian time domain are driven by the segmentation clock, which is composed of periodic signaling activities and gene expression (Hubaud and Pourquie, 2014). Cycling of the Notch, Fgf, and Wnt signaling pathways as well as alternation between activation and repression of Hes and Hes-related factor expression underlies the somite clock, sharing the principle of the circadian rhythm. Somite formation is determined by the clock and wavefront model with signaling gradients. The presomitic mesoderm is committed to a specified pattern, as rotating a portion results in the formation of inverted somites. A surgical experiment manipulating the arrangement of somites demonstrated that the somite clock is functional within a given period of development, providing another clear example of the developmental control of ultradian rhythms (Wolpert et al., 2015).

Likewise, it is important that transcriptional factors involved in the developmental processes oscillate with a proper range of time. When the unstable Hes7 protein was stabilized to have a longer half-life, without changing the repressor activity, the mouse somite segmentation was disorganized (Hirata et al., 2004). Notably, the delay between expressions was caused by the introns of the *Hes7* allele. In mice bearing the *Hes7* locus with introns removed, somites were not properly segmented, and the segmentation defects in *Hes7* null mice were rescued by introducing transgenes containing *Hes7* introns (Takashima et al., 2011). Now, with the advance of techniques such as optogenetics, methods to modulate ultradian oscillations have become more precise. Therefore, interpreting the dynamics with spatiotemporal accuracy and examining the functional role of ultradian oscillations will expand our understanding of ultradian rhythms in developmental processes (Isomura and Kageyama, 2014).

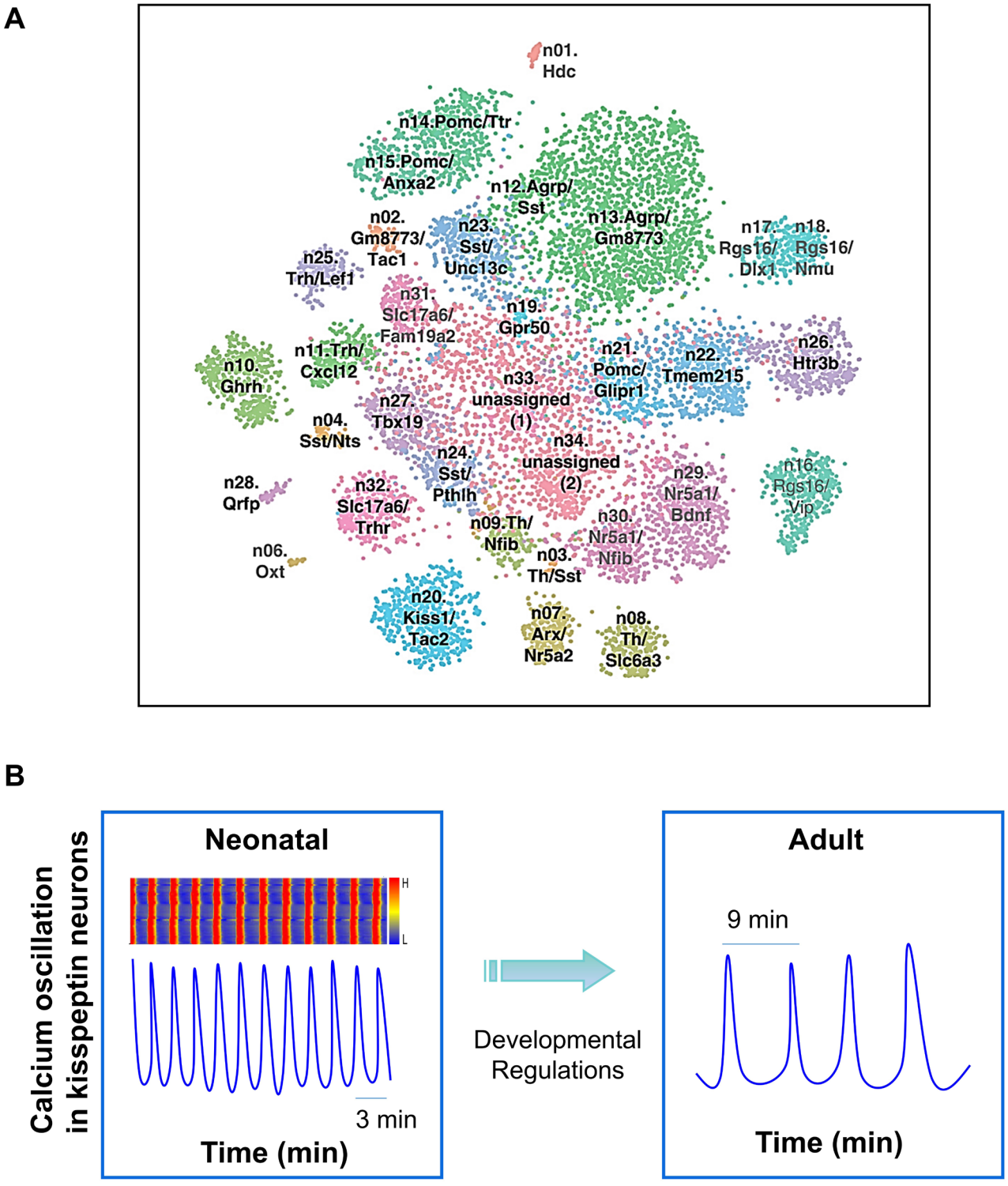

Fig. 2. Neuronal populations in the ARN and calcium oscillations in kisspeptin neurons in neonates and adults. (A) t-distributed stochastic neighbor embedding plots of neuronal cell types in the ARN are labeled according to the expression of a specific marker, or combination of marker genes, and colored according to subclustering. Clusters with gray labels likely originated from regions surrounding the ARN. In addition to kisspeptin (Kiss1) neurons, proopiomelanocortin (Pomc) and agouti-related peptide (Agrp) neurons are identified by single cell RNA-seq (Campbell et al., 2017). (B) Synchronized calcium oscillation in kisspeptin neurons from neonatal mice organotypic slice culture with a period of approximately 3 min possibly received developmental regulations and reached 9 min of calcium oscillation in adults shown by fiber photometry (modified from Clarkson et al., 2017).

PERSPECTIVES

We reviewed the characteristics of ultradian rhythms with special reference to the calcium oscillations in hypothalamic ARN kisspeptin neurons and developmental processes. Ultradian rhythms are prevalent in living organisms and can affect many physiological systems, including reproductive and developmental processes. As was the case for the circadian rhythm, revealing the mechanisms of the ultradian rhythm will fundamentally facilitate the understanding of ultradian biological processes, as well as strategies to modulate them. One of the difficulties in studying the ultradian rhythms is that they can be driven by either genomic or non-genomic mechanisms, or both. In the somite clock, a negative feedback loop incorporating transcription and translation, in combination with the cyclic activation of signaling pathways, plays a key role in its oscillation, supporting the idea of a genomic drive. Gene expression is also crucial in mediating the GnRH pulse generator and its pulsatile responses to intermittent kisspeptin treatment (Choe et al., 2013). In contrast, the calcium oscillation of ARN kisspeptin neurons is strongly regulated by ion channels and inputs via NMDA receptors. Ultradian calcium oscillation found in the SPZ-PVN region is well controlled by glutamatergic transmission. Thus, both genomic and non-genomic mechanisms are involved in regulating ultradian rhythms.

Another difficulty is that the ultradian rhythm is often overruled by circadian oscillation, complicating the analysis of time-series biological data. We speculate that technical advances in bioinformatics and genome-wide approaches will accelerate the understanding of ultradian rhythms manifested in complex biological systems. Indeed, in silico analysis of the hepatic transcriptome identified the expression of hundreds of ultradian genes, and key DNA motifs in the promoter regions of these genes (van der Veen and Gerkema, 2017). Thus, further studies should focus on both genomic and non-genomic mechanisms that drive ultradian oscillations to understand ultradian rhythms, the fundamental form of biological oscillation that regulates developmental and physiological processes.

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AUTHOR CONTRIBUTIONS

D.K. conceived and wrote the manuscript. H.K.C. consulted the design and provided feedback. K.K. supervised, provided expertise, and secured funding.

CONFLICT OF INTEREST

The authors have no potential conflicts of interest to disclose.

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REFERENCES

Belchetz, P., Plant, T., Nakai, Y., Keogh, E., and Knobil, E. (1978). Hypophysial responses to continuous and intermittent delivery of hypopthalamic gonadotropin-releasing hormone. Science *202,* 631-633.

Blum, I.D., Zhu, L., Moquin, L., Kokoeva, M.V., Gratton, A., Giros, B., and Storch, K.F. (2014). A highly tunable dopaminergic oscillator generates ultradian rhythms of behavioral arousal. eLife *3,* e05105.

Bourguignon, C. and Storch, K.F. (2017). Control of rest:activity by a dopaminergic ultradian oscillator and the circadian clock. Front. Neurol. *8,* 614.

Campbell, J.N., Macosko, E.Z., Fenselau, H., Pers, T.H., Lyubetskaya, A., Tenen, D., Goldman, M., Verstegen, A.M., Resch, J.M., McCarroll, S.A., et al. (2017). A molecular census of arcuate hypothalamus and median eminence cell types. Nat. Neurosci. *20,* 484-496.

Choe, H.K., Kim, H.D., Park, S.H., Lee, H.W., Park, J.Y., Seong, J.Y., Lightman, S.L., Son, G.H., and Kim, K. (2013). Synchronous activation of gonadotropin-releasing hormone gene transcription and secretion by pulsatile kisspeptin stimulation. Proc. Natl. Acad. Sci. U. S. A. *110,* 5677-5682.

Clarkson, J., Han, S.Y., Piet, R., McLennan, T., Kane, G.M., Ng, J., Porteous, R.W., Kim, J.S., Colledge, W.H., Iremonger, K.J., et al. (2017). Definition of the hypothalamic GnRH pulse generator in mice. Proc. Natl. Acad. Sci. U. S. A. *114,* E10216-E10223.

Clarkson, J. and Herbison, A.E. (2006). Postnatal development of kisspeptin neurons in mouse hypothalamus; sexual dimorphism and projections to gonadotropin-releasing hormone neurons. Endocrinology *147,* 5817-5825.

Cortassa, S.D.C., Aon, M.A., Aon, J.C., Iglesias, A.A., and Lloyd, D. (2011). An Introduction to Metabolic and Cellular Engineering (2nd Edition) (Singapore: World Scientific Publishing Company).

Dibner, C., Schibler, U., and Albrecht, U. (2010). The mammalian circadian timing system: organization and coordination of central and peripheral clocks. Annu. Rev. Physiol. *72,* 517-549.

Edgar, R.S., Green, E.W., Zhao, Y., van Ooijen, G., Olmedo, M., Qin, X., Xu, Y., Pan, M., Valekunja, U.K., Feeney, K.A., et al. (2012). Peroxiredoxins are conserved markers of circadian rhythms. Nature *485,* 459-464.

Fu, L.Y. and van den Pol, A.N. (2010). Kisspeptin directly excites anorexigenic proopiomelanocortin neurons but inhibits orexigenic neuropeptide Y cells by an indirect synaptic mechanism. J. Neurosci. *30,* 10205-10219.

Gachon, F., Nagoshi, E., Brown, S.A., Ripperger, J., and Schibler, U. (2004). The mammalian circadian timing system: from gene expression to physiology. Chromosoma *113,* 103-112.

Gill, J.C., Navarro, V.M., Kwong, C., Noel, S.D., Martin, C., Xu, S., Clifton, D.K., Carroll, R.S., Steiner, R.A., and Kaiser, U.B. (2012). Increased neurokinin B (Tac2) expression in the mouse arcuate nucleus is an early marker of pubertal onset with differential sensitivity to sex steroid-negative feedback than Kiss1. Endocrinology *153,* 4883-4893.

Goldbeter, A. (2008). Biological rhythms: clocks for all times. Curr. Biol. *18,* R751-R753.

Hastings, M.H., Maywood, E.S., and Brancaccio, M. (2018). Generation of circadian rhythms in the suprachiasmatic nucleus. Nat. Rev. Neurosci. *19,* 453-469.

Herbison, A.E. (2016). Control of puberty onset and fertility by gonadotropin-releasing hormone neurons. Nat. Rev. Endocrinol. *12,* 452-466.

Herbison, A.E. (2018). The gonadotropin-releasing hormone pulse generator. Endocrinology *159,* 3723-3736.

Hirata, H., Bessho, Y., Kokubu, H., Masamizu, Y., Yamada, S., Lewis, J., and Kageyama, R. (2004). Instability of Hes7 protein is crucial for the somite

segmentation clock. Nat. Genet. *36,* 750-754.

Hubaud, A. and Pourquie, O. (2014). Signalling dynamics in vertebrate segmentation. Nat. Rev. Mol. Cell Biol. *15*, 709-721.

Imayoshi, I., Isomura, A., Harima, Y., Kawaguchi, K., Kori, H., Miyachi, H., Fujiwara, T., Ishidate, F., and Kageyama, R. (2013). Oscillatory control of factors determining multipotency and fate in mouse neural progenitors. Science *342,* 1203-1208.

Isomura, A. and Kageyama, R. (2014). Ultradian oscillations and pulses: coordinating cellular responses and cell fate decisions. Development *141,* 3627-3636.

Israel, J.M., Le Masson, G., Theodosis, D.T., and Poulain, D.A. (2003). Glutamatergic input governs periodicity and synchronization of bursting activity in oxytocin neurons in hypothalamic organotypic cultures. Eur. J. Neurosci. *17*, 2619-2629.

Kageyama, R., Niwa, Y., and Shimojo, H. (2009). Rhythmic gene expression in somite formation and neural development. Mol. Cells *27*, 497-502.

Kalafatakis, K., Russell, G.M., Harmer, C.J., Munafo, M.R., Marchant, N., Wilson, A., Brooks, J.C., Durant, C., Thakrar, J., Murphy, P., et al. (2018). Ultradian rhythmicity of plasma cortisol is necessary for normal emotional and cognitive responses in man. Proc. Natl. Acad. Sci. U. S. A. *115*, E4091-E4100.

Kim, D., Jang, S., Kim, J., Park, I., Ku, K., Choi, M., Lee, S., Heo, W.D., Son, G.H., Choe, H.K., et al. (2020). Kisspeptin neuron-specific and self-sustained calcium oscillation in the hypothalamic arcuate nucleus of neonatal mice: regulatory factors of its synchronization. Neuroendocrinology 2020 Jan 15 [Epub]. https://doi.org/10.1159/000505922

Kumar, D., Periasamy, V., Freese, M., Voigt, A., and Boehm, U. (2015). In utero development of kisspeptin/GnRH neural circuitry in male mice. Endocrinology *156*, 3084-3090.

Lamont, E.W. and Amir, S. (2010). Circadian and ultradian clocks/rhythms. In Encyclopedia of Behavioral Neuroscience, G.F. Koob, M.L. Moal, and R.F. Thompson, eds. (Oxford: Academic Press), pp. 257-261.

Lee, J., Chun, S.K., Son, G.H., and Kim, K. (2015). Sumoylation of Hes6 regulates protein degradation and Hes1-mediated transcription. Endocrinol. Metab. (Seoul) *30*, 381-388.

Lightman, S.L. and Conway-Campbell, B.L. (2010). The crucial role of pulsatile activity of the HPA axis for continuous dynamic equilibration. Nat. Rev. Neurosci. *11*, 710-718.

Lloyd, D., Aon, M.A., and Cortassa, S. (2001). Why homeodynamics, not homeostasis? ScientificWorldJournal *1*, 133-145.

Lopp, S., Navidi, W., Achermann, P., LeBourgeois, M., and Diniz Behn, C. (2017). Developmental changes in ultradian sleep cycles across early childhood. J. Biol. Rhythms *32*, 64-74.

McCartney, C.R. and Marshall, J.C. (2014). Neuroendocrinology of reproduction. In Yen & Jaffe's Reproductive Endocrinology, 7th ed., J.F. Strauss and R.L. Barbieri, eds. (Philadelphia, Elsevier Saunders), pp. 3-26.

McGinnis, G.R. and Young, M.E. (2016). Circadian regulation of metabolic homeostasis: causes and consequences. Nat. Sci. Sleep *8*, 163-180.

Muller, T.D., Nogueiras, R., Andermann, M.L., Andrews, Z.B., Anker, S.D., Argente, J., Batterham, R.L., Benoit, S.C., Bowers, C.Y., Broglio, F., et al. (2015). Ghrelin. Mol. Metab. *4*, 437-460.

Navarro, V.M., Ruiz-Pino, F., Sanchez-Garrido, M.A., Garcia-Galiano, D., Hobbs, S.J., Manfredi-Lozano, M., Leon, S., Sangiao-Alvarellos, S., Castellano, J.M., Clifton, D.K., et al. (2012). Role of neurokinin B in the control of female puberty and its modulation by metabolic status. J. Neurosci. *32*, 2388-2397.

Pinilla, L., Aguilar, E., Dieguez, C., Millar, R.P., and Tena-Sempere, M. (2012). Kisspeptins and reproduction: physiological roles and regulatory mechanisms. Physiol. Rev. *92*, 1235-1316.

Ribeiro, A.B., Leite, C.M., Kalil, B., Franci, C.R., Anselmo-Franci, J.A., and Szawka, R.E. (2015). Kisspeptin regulates tuberoinfundibular dopaminergic neurones and prolactin secretion in an oestradiol-dependent manner in male and female rats. J. Neuroendocrinol. *27*, 88-99.

Seminara, S.B., Messager, S., Chatzidaki, E.E., Thresher, R.R., Acierno, J.S., Jr, Shagoury, J.K., Bo-Abbas, Y., Kuohung, W., Schwinof, K.M., Hendrick, A.G., et al. (2003). The GPR54 gene as a regulator of puberty. N. Engl. J. Med. *349*, 1614-1627.

Smedler, E. and Uhlen, P. (2014). Frequency decoding of calcium oscillations. Biochim. Biophys. Acta *1840*, 964-969.

Son, G.H., Chung, S., Choe, H.K., Kim, H.D., Baik, S.M., Lee, H., Lee, H.W., Choi, S., Sun, W., Kim, H., et al. (2008). Adrenal peripheral clock controls the autonomous circadian rhythm of glucocorticoid by causing rhythmic steroid production. Proc. Natl. Acad. Sci. U. S. A. *105*, 20970-20975.

Sonnen, K.F., Lauschke, V.M., Uraji, J., Falk, H.J., Petersen, Y., Funk, M.C., Beaupeux, M., François, P., Merten, C.A., and Aulehla, A. (2018). Modulation of phase shift between Wnt and Notch signaling oscillations controls mesoderm segmentation. Cell *172*, 1079-1090.e12.

Szawka, R.E., Ribeiro, A.B., Leite, C.M., Helena, C.V., Franci, C.R., Anderson, G.M., Hoffman, G.E., and Anselmo-Franci, J.A. (2010). Kisspeptin regulates prolactin release through hypothalamic dopaminergic neurons. Endocrinology *151*, 3247-3257.

Takahashi, J.S. (2017). Transcriptional architecture of the mammalian circadian clock. Nat. Rev. Genet. *18*, 164-179.

Takashima, Y., Ohtsuka, T., Gonzalez, A., Miyachi, H., and Kageyama, R. (2011). Intronic delay is essential for oscillatory expression in the segmentation clock. Proc. Natl. Acad. Sci. U. S. A. *108*, 3300-3305.

van der Veen, D.R. and Gerkema, M.P. (2017). Unmasking ultradian rhythms in gene expression. FASEB J. *31*, 743-750.

Voliotis, M., Li, X.F., De Burgh, R., Lass, G., Lightman, S.L., O'Byrne, K.T., and Tsaneva-Atanasova, K. (2019). The origin of GnRH pulse generation: an integrative mathematical-experimental approach. J. Neurosci. *39*, 9738-9747.

Wolpert, L., Tickle, C., and Arias, A.M. (2015). Principles of Development (Oxford: Oxford University Press).

Wu, Y.E., Enoki, R., Oda, Y., Huang, Z.L., Honma, K.I., and Honma, S. (2018). Ultradian calcium rhythms in the paraventricular nucleus and subparaventricular zone in the hypothalamus. Proc. Natl. Acad. Sci. U. S. A. *115*, E9469-E9478.

Yates, F.E. and Yates L.B. (2008). Ultradian rhythms as the dynamic signature of life. In Ultradian Rhythms from Molecules to Mind, D. Lloyd and E. Rossi, eds. (Dordrecht, Netherlands: Springer Science+Business Media B.V.), pp. 249-260.

Yildiz, B., Suchard, M., Wong, M., McCann, S., and Licinio, J. (2004). Alterations in the dynamics of circulating ghrelin, adiponectin, and leptin in human obesity. Proc. Natl. Acad. Sci. U. S. A. *101*, 10434-10439.