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Core clock gene, *Bmal1*, is required for optimal second-level interval production

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ABSTRACT

Perception and production of second-level temporal intervals are critical in several behavioral and cognitive processes, including adaptive anticipation, motor control, and social communication. These processes are impaired in several neurological and psychological disorders, such as Parkinson's disease and attention-deficit hyperactivity disorder. Although evidence indicates that second-level interval timing exhibit circadian patterns, it remains unclear whether the core clock machinery controls the circadian pattern of interval timing. To investigate the role of core clock molecules in interval timing capacity, we devised a behavioral assay called the interval timing task to examine prospective motor interval timing ability. In this task, the mouse produces two separate nose pokes in a pretrained second-level interval to obtain a sucrose solution as a reward. We discovered that interval perception in wild-type mice displayed a circadian pattern, with the best performance observed during the late active phase. To investigate whether the core molecular clock is involved in the circadian control of interval timing, we employed *Bmal1* knockout mice (BKO) in the interval timing task**.** The interval production of BKO did not display any difference between early and late active phase, without reaching the optimal interval production level observed in wild-type. In summary, we report that the core clock gene *Bmal1* is required for the optimal performance of prospective motor timing typically observed during the late part of the active period.

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Introduction

Animals possess an intrinsic sense of time, spanning sub-seconds, seconds, minutes, hours, days, and even years. This temporal awareness enables them to adapt to environmental changes and prepare for their biological needs in advance. While the spectrum of temporal events is continuously distributed across these orders, distinct biological timing mechanisms are responsible for specific time ranges. The most extensively studied biological timekeeping mechanism is the circadian clock, which governs many daily fluctuations in physiological and behavioral processes (Takahashi 2016; Lee and Kim 2012). In addition, recent research suggests that the trajectory pattern of neuronal activation plays a role in governing timing at the sub-second to minute level (Tsao et al. 2022; Paton and Buonoman 2018). This impacts various brain functions, including adaptive anticipation, motor control, and social communication (Mioni et al. 2021; Warda and Khan 2022; Aydoğan et al. 2023). While these timing systems operate through distinct mechanisms, the circadian rhythm can influence the performance of the second-level timing system. For instance, mice exhibit greater accuracy and precision in second-level intervals at night compared to daytime (Agostino et al. 2011).

Circadian rhythms are self-sustaining endogenous molecular timing systems that organize biological and cognitive processes to predict environmental and biological needs occurring daily. The transcription and translational feedback loop serve as the driving force behind the circadian rhythm (Takahashi 2016), alongside post-transcriptional and post-translational regulation (Lee and Kim 2012). Specifically, the transcription factors CLOCK and BMAL1 form a heterodimer, activating the transcription of genes containing the E-box *cis*-element in their promoters. The target genes of CLOCK and BMAL1 include *Per*s and *Cry*s, which act as transcriptional repressors that suppress E-box-mediated transcription. This constitutes a negative feedback loop at the core of the transcription-translational loop. An additional layer of negative feedback loops, involving gene networks operating through

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RRE and D-box, interlock with the E-box-mediated loop, forming the genetic machinery known as the circadian clock. The circadian clock finely tunes the rhythmicity of oscillatory gene expression across the entire gene network for approximately 24 h (Takahashi 2016). The circadian clock's role extends beyond rhythm generation, governing various biological processes through transcriptional regulations and post-transcriptional modifications (Bass and Lazar 2016; Kim et al. 2023). Circadian clock gene-knockout mice, such as *Bmal1*$^{-/-}$, *Cry1*$^{-/-}$*Cry2*$^{-/-}$, and *Per1*$^{-/-}$*Per2*$^{-/-}$ play pivotal roles in understanding numerous circadian rhythm-controlled biological processes.

The potential interactions between the circadian clock and other timing systems operating on different timescales remain unknown. Mutant mouse studies have suggested that genetic mutations causing alterations in the circadian period are correlated with changes in interval timing production (Balzani et al. 2016; Maggi et al. 2017). For instance, the After-hour (*Afh*) mutant, which exhibits a longer circadian period, also produces longer intervals. Conversely, the *Zfhx3* mutant, with a shorter circadian period, generates shorter intervals compared to the wild type. It is intriguing to note that the timing intervals of core clock gene mutants, such as *Cry1*$^{-/-}$*Cry2*$^{-/-}$ or *Clock*$^{-/-}$, remain unaffected (Cordes and Gallistel 2008; Papachristos et al. 2011), warranting further investigation. In this study, we employed a prospective motor timing test to assess the interval timing capacity of wild-type and *Bmal1*$^{-/-}$ mice.

Materials and methods

Mice

Adult male C57BL/6J mice were used in this study. Mice were housed under a 12-h light:12-h dark regime (light on from 10:00–22:00) with *ad libitum* access to food and water. During the behavioral tasks, sucrose was provided exclusively as a reward for the interval timing task, while food remained freely accessible at the bottom of their cages. We closely monitored the health status of the mice daily and observed no signs of dehydration-related issues. *Bmal1* knockout mice (Bunger et al. 2000) were generously provided by Marina Antoch (Roswell Park Cancer Institute, Buffalo, NY, USA) and Karyn Esser (University of Kentucky, Lexington, KY, USA). Data were collected from four wild-type mice and three *Bmal1* knockout mice. All animal procedures were approved and conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of Daegu Gyeongbuk Institute of Science and Technology (DGIST-IACUC-23083111-0001).

Behavioral rig

All behavioral assays were performed using a custom behavior rig controlled by an Arduino Mega2560 board (Arduino, Ivrea, Italy). The rig was constructed as a 200 × 200 × 200 mm cube using acrylic sheets (Acrylmall, Incheon, South Korea). A nose-poking hole, equipped with a metallic nozzle in the center, was incorporated into one of the walls. An IR sensor attached to the metallic nozzle detected the head poking of the mouse and relayed this information to the Arduino. A 10% v/v sucrose solution was dispensed as a reward into the poking hole through a valve controlled by the Arduino. The rig also featured a speaker to produce guidance beeps during training. A list of all the components used to assemble the rig can be found in Table 1.

Interval timing task procedure

The mice were housed in a behavioral rig throughout the experimental period and actively engaged in the task. The behavioral rig was maintained within a temperature- and light-controlled chamber following a 12:12 LD cycle. The interval timing task consisted of three phases: a fixed-ratio schedule of 2 (FR2), pretraining, and interval production. During the FR2 phase, a sucrose reward was provided every two pokes, regardless of the interval between pokes. This phase lasted for two days. The second phase was the pretraining, during which mice were trained to initiate a second poke after a specified waiting period following the first poke. A beep sound alerted the mice to the task five seconds after the first poke. Poking before the cue was

Table 1. Part information used to build the behavior rig.

Resource	Source	Identifier
Behavior control system		
Photointerrupter	SHARP (Hsinchu, Taiwan)	GP1S093HCZ0F
GF063P-501(500 ohm)	TOCOS (Tokyo, Japan)	P001821995
GF063P-204(200 kohm)	TOCOS (Tokyo, Japan)	P001822005
Diode (1N4001)	JGD (Jinan, China)	EPX33G6J
Single 5 V relay	STACKPOLE (Saint Marys, PA, USA)	Jqc-3ff-s-z
Breadboard (solderless), 10 × 30	WANJIE (Zhejiang, China)	ELB-80T
Adafruit Assembled data logging shield for arduino	Adafruit (New York, USA)	P0000BHN
16 × 2 Character LCD Display Shield	Adafruit (New York, USA)	Rs-004147
Arduino Mega2560 R3	Arduino (Ivrea, Italy)	EPX67L3A
Reward delivery system		
Stainless steel tubing	Lklab Korea (Namyangju-si, Gyeonggi-do, South Korea)	T23-184-07
Solenoid valve	Skoocom (Shenzhen, China)	SC0829GW
Sound delivery system		
Gravity digital speaker module	DFRobot (Shanghai, China)	N/A

considered premature while poking within 0–5 s of the cue (the target time) was considered correct. If the mouse poked 5 s after the cue, it was considered a late response. A sucrose reward was given only for correct trials, and if the percentage of correct trials exceeded 35%, the mice proceeded to the third phase. The third phase, interval production, was identical to pretraining, except for the cue. After the first poke, a beep was given, but no further auditory cues were provided. In this phase, the mice relied on their internal timing system to initiate the second poke. A sucrose reward was delivered when the mouse made its second poke between 5–10 s after the first poke. The success rate was calculated as the number of rewarded trials (correct) divided by the total number of trials (correct, premature, and late). The mice initiated FR2 at the age of 7 weeks and conducted behavioral experiments until the age of 14 weeks. In the target time alteration task, target time is increased by 1 s once the success rate exceeded 35% in the previous 200 trials. For the circadian analysis, data were collected from mice that met the threshold specified below. If the mode values of the produced intervals in every hundred-trial set fell between 4.5 and 5.5 s for 2–3 consecutive days, the mouse was considered to have passed the threshold. The trials required to pass the threshold for the wild-type and *Bmal1* knockouts were not significantly different and as follows: 6,000, 6,700, 5,100, and 6,300 for the wild-type, and 6,500, 3,900, and 5,400 for the *Bmal1* knockouts.

Statistical analysis and schematic drawing

The data were analyzed using paired t-tests, one-way ANOVA, and two-way ANOVA, as specified in each figure. Statistical analysis was performed using GraphPad Prism software. Significant differences were denoted by p-values < 0.05 and are indicated as follows: $*p < 0.05$; $**p < 0.005$; $***p < 0.001$. A part of schematics is prepared using Scidraw (https://scidraw.io/).

Results

Behavioral assessment of interval timing task

To investigate the second-level timing system in mice, we first established a second-level interval timing task (Figure 1A). Once the mouse pokes the poke hole, a brief beep signals the start of the task. The mouse then waits for a voluntary period and produces a second poke. If the interval between the first and second pokes (poke interval) is shorter than 5 s, the trial is classified as premature**.** A trial with poke interval between 5 and 10 s is counted as correct. If the poke interval is longer than 10 s, it is considered late. Sucrose solution is administered to mice only in the correct trials, while either reward or punishment is administered in the premature and late trials. After a certain amount of trial and error, the mice produce a second poke during the target interval (between 5 and 10 s after the first poke) (Figure 1B). While the mice predominantly produce premature poking in the initial trials, the mode of the second poke every hundred trials gradually increases to reach the targeted interval after several thousand trials (Figure 1C). We also calculate the success rate of the interval production. The success rate is defined as the number of correct trials divided by the total number of trials (premature, correct, and late trials). Similarly, the success rate gradually increases and then plateaus at 40 percent (Figure 1D). The results show that the behavioral setup employed can measure the second-level interval timing of the mouse.

We then investigated whether mice could adjust the interval duration when the target interval was implicitly shifted. To address this question, we changed the target time from 5 s to 10 s in a one-second step when the success rate of the mouse approached a plateau (Figure 2). The change in the target interval is not explicitly signaled to the mouse, for example, through a beeping sound. The mouse adjusts interval production based on reinforcement, although each mouse exhibits an occasional period of enriched premature trials, possibly because of the increased task difficulty posed by implicit changes in the target duration. The heatmaps of the poke interval in two representative mice consistently show that the mice actively adjust the duration of the interval. These results demonstrate the second-level interval production capacity of the mice, which is in line with previous studies based on different behavioral protocols (Agostino et al. 2011).

Circadian pattern of interval timing production

Before investigating the circadian pattern of interval timing, we first assessed the stability of the interval timing task. During the interval production phase, the success rate reached a plateau after approximately five thousand trials, with a gradual increase in the production interval over this period (Figure 1B-D). If a mouse consistently exhibited a daily mode interval value between 4.5 and 5.5 s for two to three consecutive days, we considered the interval production capacity of the mouse to be stabilized, and the next day was counted as day one. Over the course of the five-day analysis, the daily mode value of interval timing

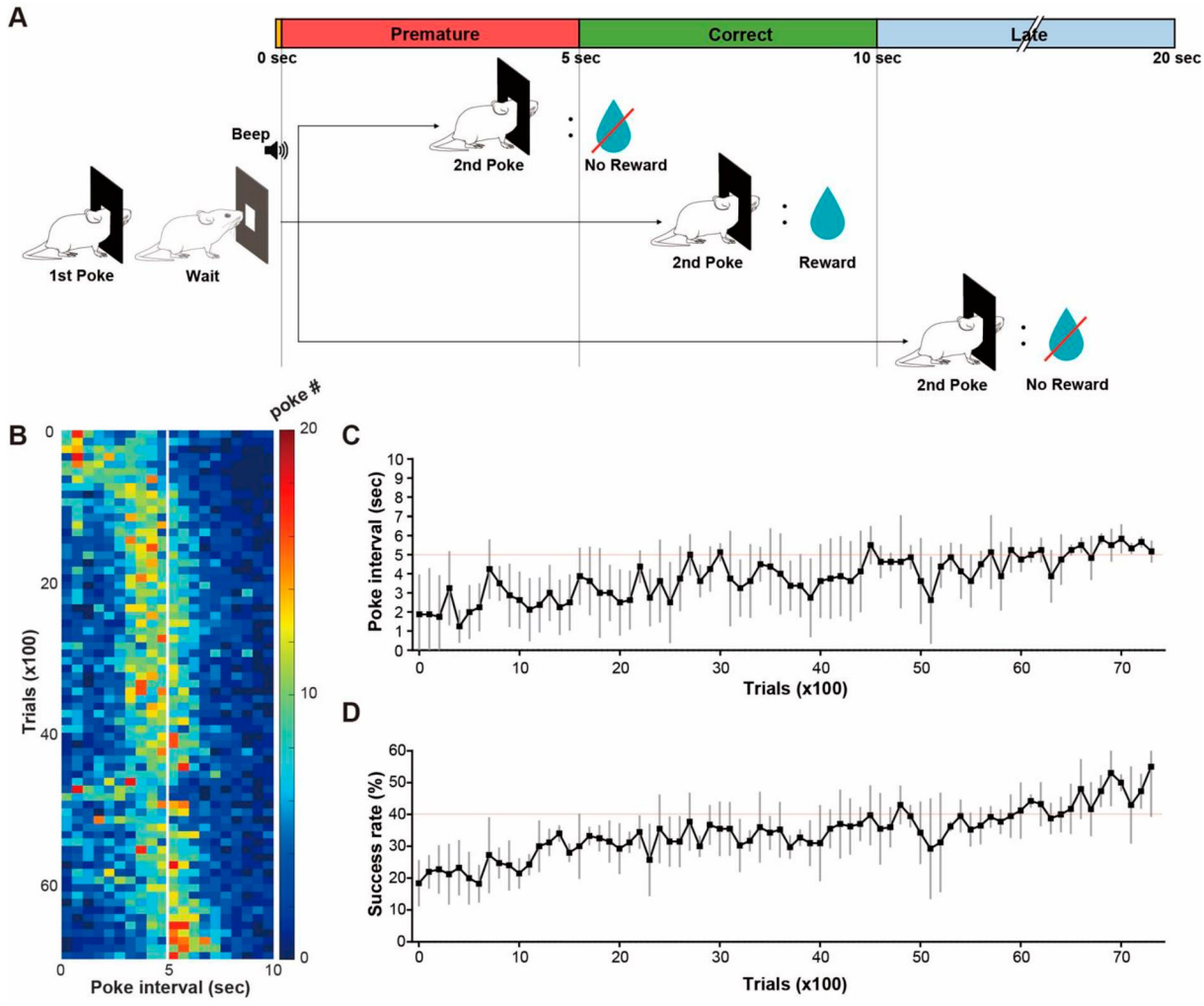

Figure 1. Second-level interval production in wildtype mice. **A**. Schematic of the interval production phase in the interval timing task. A mouse initiates the trial by poking the licking port. After the first poke, a beep sound is given to alarm the initiation of interval. The mouse relies on the internal timing system to determine when to produce the second poke. If the second poke of the mouse lies within the target duration (5-10 sec), the mouse can obtain sucrose solution droplet as a reward. Premature (0-5 sec) or late (10-20 sec) trial is not rewarded. **B.** A representative heatmap showing poke intervals. White line: the onset of the correct target duration. The number of pokes at each pixel is shown at the right color table. **C.** Mode value of poke interval calculated in 100-trial bin. Red line: the onset of the correct target duration. **D.** Average success rate calculated in 100-trial bin. Success rate is increased as training progressed. Success rate is calculated as follows: [The number of correct trials] / ([The number of premature trials] + [The number of correct trials] + [The number of late trials]). Red line: 40% guideline. **C**-**D**: Data are presented as mean ± one standard deviation. n = 4 mice.

remained stable around the targeted time duration onset (Figure 3A). The mice consistently showed similar success rates across these five days (Figure 3B). Furthermore, the number of total drinks per day remained consistent during this analysis period, suggesting that the motivation of the mouse was maintained throughout (Figure 3C). As the daily patterns of mode produced interval values, success rates, and the number of drinks were largely consistent after day one, we averaged the data from days one to five and analyzed the diurnal pattern. It was observed that the mice obtained rewards predominantly during the active phase (ZT12-24) (Figure 3D) when the motivation for thirst-quenching behavior was at its peak (Gizowski et al. 2016). Our focus then shifted to interval production during the active phase to examine time-dependent changes in the quality of interval timing behavior. During the active phase, the number of sucrose rewards obtained was slightly higher in the early section of the active phase, although without statistical significance. When comparing the success rate of the early active phase (ZT12-16) with that of the late active phase (ZT20-24), it became evident that the success rate during the late active phase was significantly

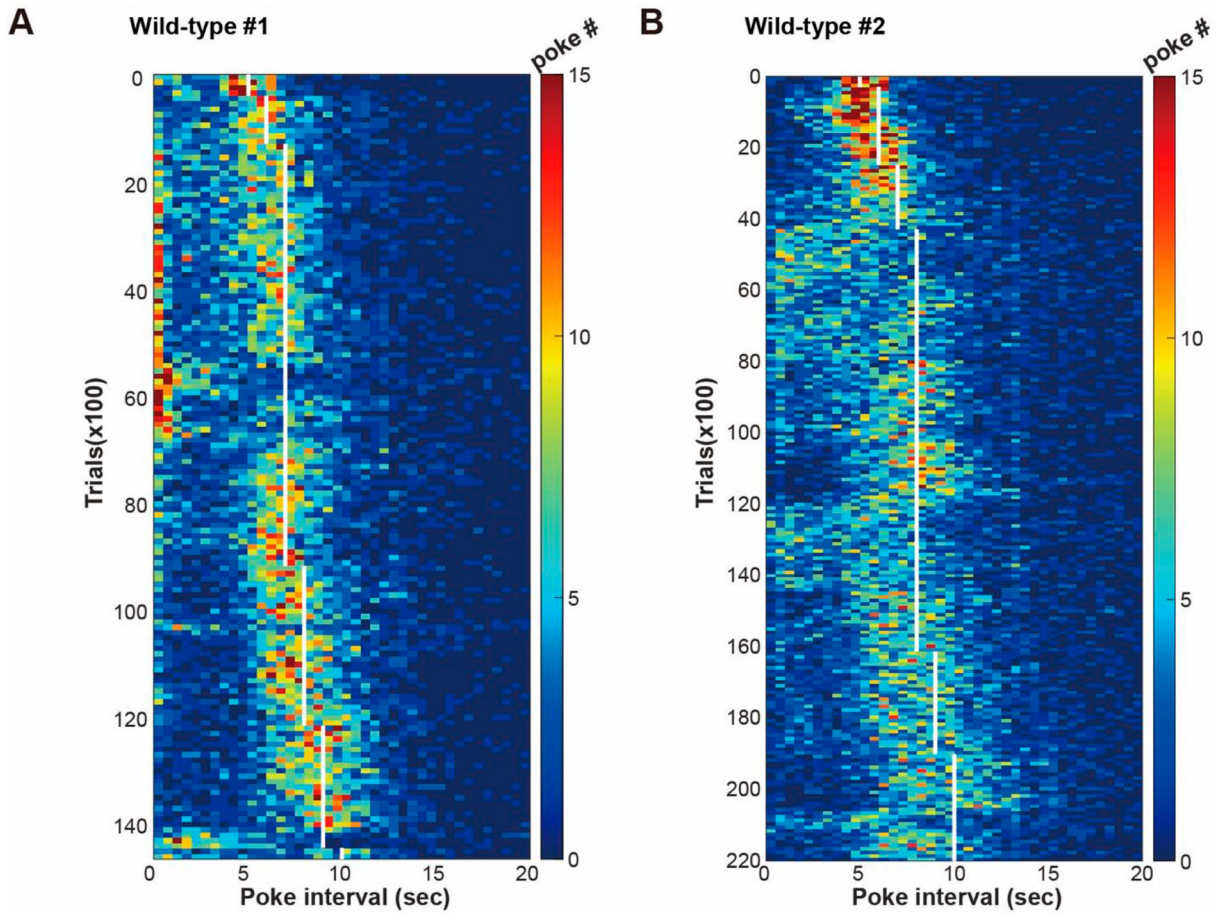

Figure 2. Poke interval is malleable by target time alteration. **A, B**. Heatmaps of poke intervals from two distinct wild-type mice during target time alteration. The number of pokes at each pixel is shown at the right color table. White line: the onset of target duration.

higher (Figure 3E). To delve deeper, we examined the distribution of the number of trials between ZT12-16 and ZT20-24. It was observed that the number of produced intervals appeared to increase during the time between the pre-target and early target durations (Figure 3F). These trials were classified into three groups: premature, correct, and late (Figure 3G). While the proportion of premature trials was higher in ZT12-16 than in ZT20-24, the proportion of correct trials was higher in ZT20-24, contributing to a higher correct rate in this time zone. The proportion of late trials remained similar in both periods. These results indicate that the interval timing performance of mice was more accurate in ZT20-24 due to a reduced premature interval production rate.

Core clock gene, Bmal1*, is required for the diurnal variation in interval timing*

To investigate whether core clock genes are involved in diurnal interval timing patterns, we utilized *Bmal1* knockout (BKO) mice (Bunger et al. 2000). BMAL1 is an essential transcription factor in the molecular clock. The genetic knockout of *Bmal1* resulted in arrhythmic locomotor behavior and several other circadian phenotypes, as reported by Ripperger et al. (2011), Jiang et al. (2022), and Schiaffino et al. (2016). This model system provides an excellent platform for examining the regulation of behavioral and physiological functions by the molecular clockwork. We trained three BKO using an interval timing task. The knockout animals consistently acquired the interval timing task (Figure 4A). The mode interval duration and success rate over 100 trials gradually increased, plateauing at approximately 5 s and 40%, respectively (Figure 4B and C), similar to the wild-type (Figure 1C). To assess whether interval production in BKO mice remained stable across days after reaching this plateau, we examined the mode value of the produced interval, success rate, and the number of rewards obtained. Our data indicated that these parameters remained stable for 5 days after reaching the plateau, with only slight and non-significant fluctuations (Figure 4D-F). It is of note that the daily numbers of rewards obtained are similar between WT and BKO

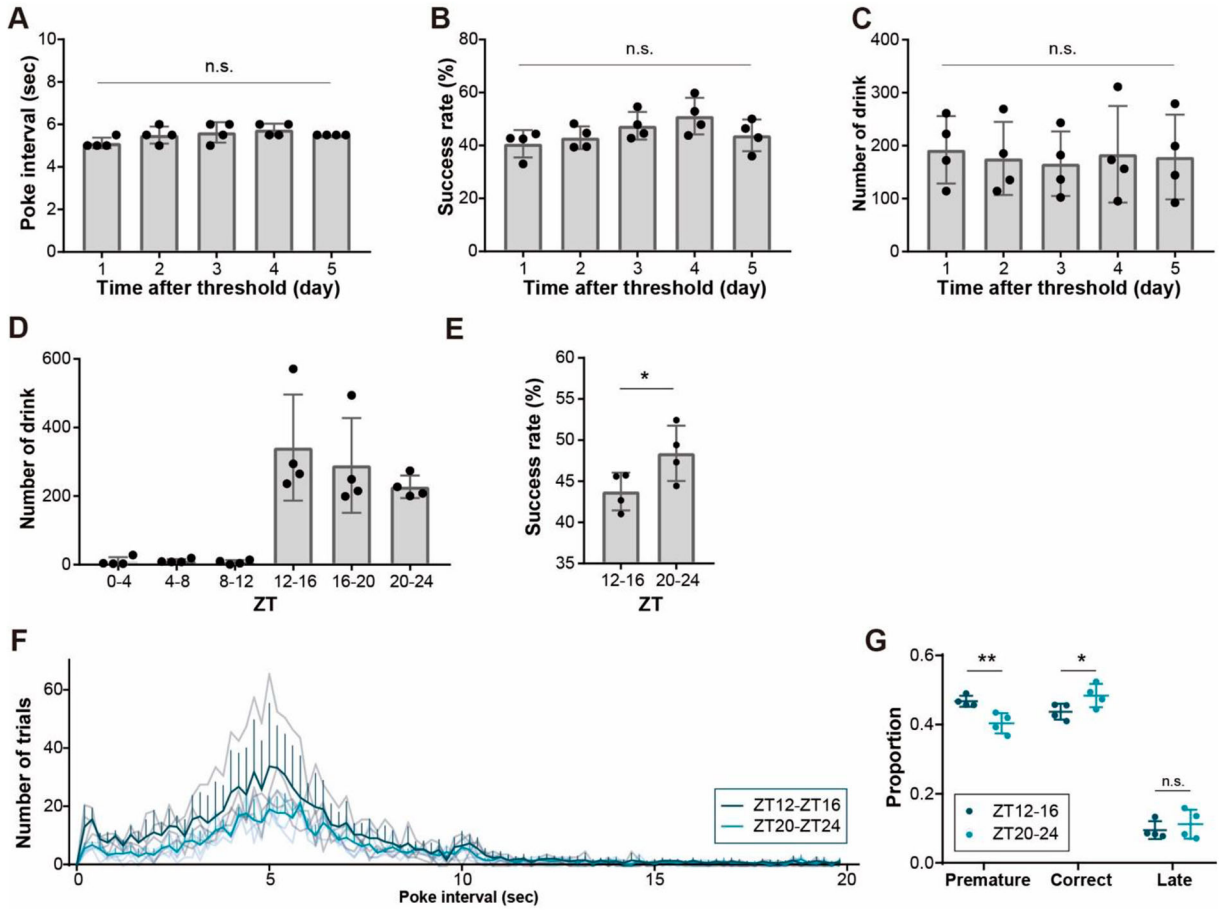

Figure 3. Diurnal pattern of interval production. **A**. Daily mode of poke interval during 5 days after reaching the threshold. $p = 0.2392$ by repeated measures one-way ANOVA. **B**. Daily average of success rate. $p = 0.1832$ by repeated measures one-way ANOVA. **C**. Daily average of obtained rewards. $p = 0.7080$ by repeated measures one-way ANOVA. **D**. The number of obtained rewards at different time of day. ZT: zeitgeber time. **E**. Average success rate at ZT12-16 and ZT20-24. *: $p < 0.05$ by paired t test. **F**. Distribution of poke intervals at ZT12-16 and ZT20-24. **G**. Proportion of trials at two time points. **: $p < 0.01$; *: $p < 0.05$ by Bonferroni's multiple comparisons test following two-way ANOVA. For **A**-**G**, Data are presented as mean with SD. n = 4 mice.

(Figure 3C and 4F), suggesting that BKO does not alter reward-seeking behavior itself. This is in accordance with the previous reports that sucrose consumption is not affected by cell type-specific *Bmal1* knockout (Landgraf et al. 2016; Zavalia et al. 2021). We also analyzed the circadian patterns of interval production in BKO mice during different phases of the day. In contrast to the distinct biphasic distribution of drink consumption observed in wild-type (WT) mice (Figure 3D), BKO mice obtained sucrose rewards even during the light-on phase, with more sucrose rewards acquired during the nighttime (Figure 4G). We further compared the success rates between the ZT12-16 and ZT20-24, during which a significant difference in success rate was observed in the WT group (Figure 3E). Notably, the success rates were similar at both time points in the BKO group (Figure 3H). We then examined the distribution of produced intervals, finding that the distributions for BKO mice at ZT12-16 and ZT20-24 largely overlapped (Figure 3I). Moreover, the classification of all trials into premature, correct, and late trials did not reveal any differences between ZT12-16 and ZT20-24 (Figure 4J). Our results demonstrate that *Bmal1* knockout mice are capable of producing second-level intervals and highlight the essential role of *Bmal1* in shaping the daily pattern of interval production, even under regular 12-hour light-dark conditions.

Comparison of interval timing performance in WT and BKO

So far, we have observed diurnal variations in interval timing performance in wild-type mice, a phenomenon disrupted in mice lacking the circadian core clock gene *Bmal1*. In order to gain a more comprehensive understanding of the interval timing performance in BKO, we conducted a re-analysis of the poke intervals of WT and BKO mice at two distinct time periods, ZT12-16

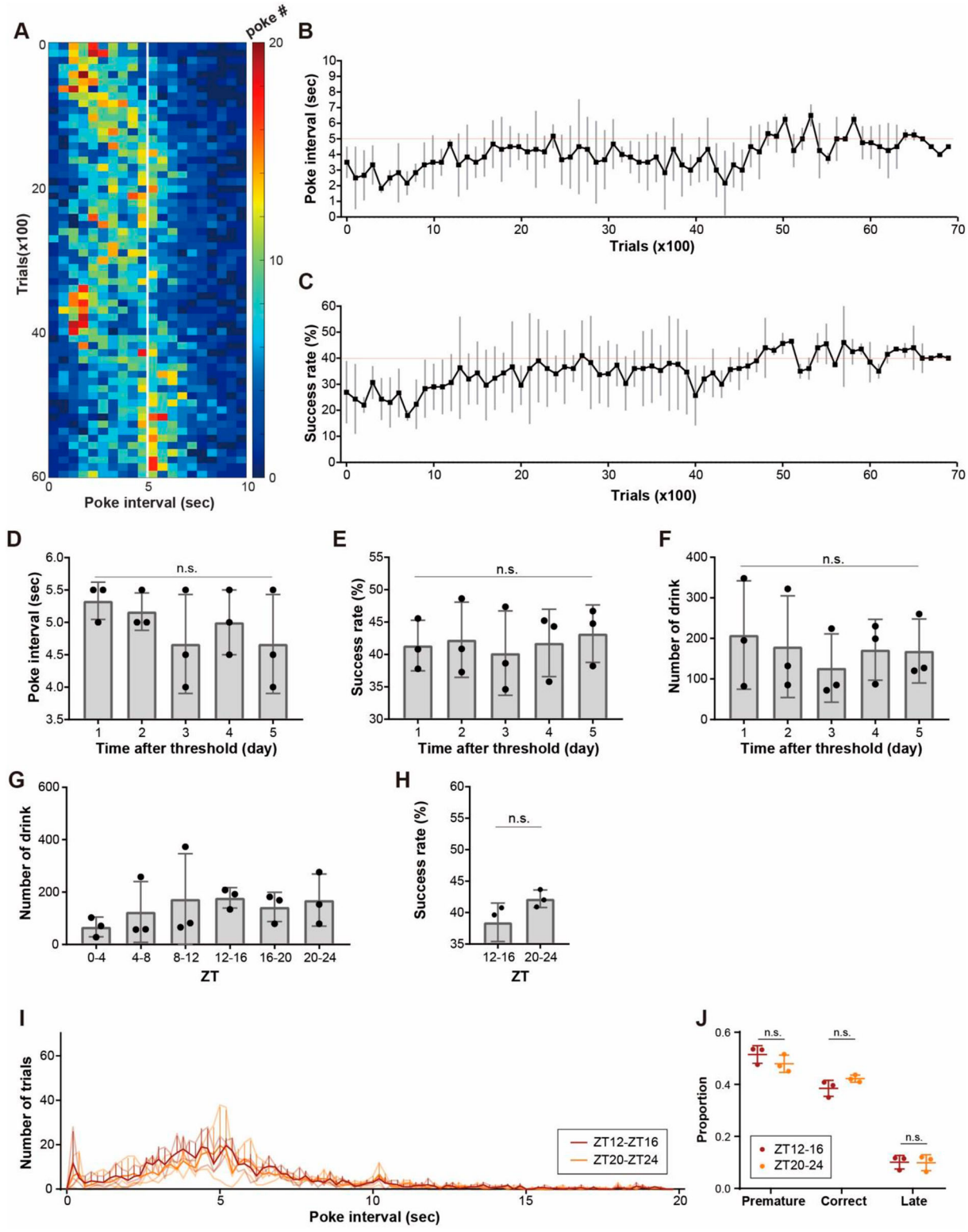

Figure 4. *Bmal1* KO does not show the different poking performance between early and late active phase. **A**. A representative heatmap of produced interval of *Bmal1*$^{-/-}$. The number of pokes at each pixel is shown at the right color table. White line: the onset of target duration. **B**. Mode of poke intervals calculated in 100-trial bin. Red line: the onset of target duration. **C**. Success rate calculated in 100-trial bin. Red line: 40% guideline. **D**. Daily Mode of poke intervals. $p = 0.2304$ by repeated measures one-way ANOVA. **E**. Daily success rate. $p = 0.6935$ by repeated measures one-way ANOVA. **F**. The number of obtained rewards. $p = 0.3417$ by repeated measures one-way ANOVA. **G**. The number of obtained rewards at different time of day. ZT: zeitgeber time. **H**. Average success rate at ZT12-16 and ZT20-24. $p = 0.1600$ by paired t test. **I**. Distribution of poke intervals at ZT12-16 and ZT20-24. **J**. Proportion of trials at two time points. $p > 0.05$ by Bonferroni's multiple comparisons test following two-way ANOVA. For **B**-**J**, Data are presented as mean with SD. n = 3 mice.

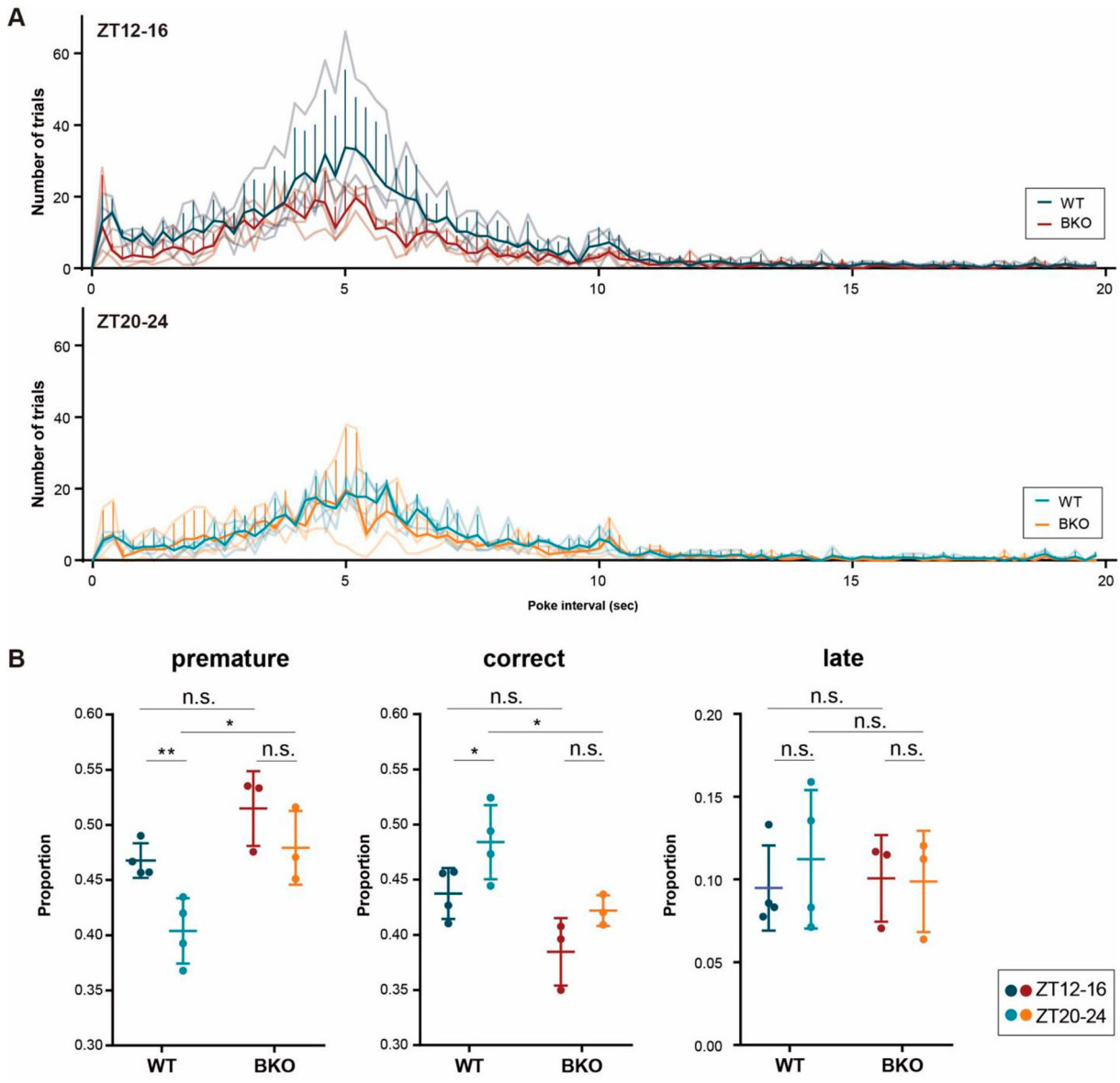

Fig. 5. Increased correct performance at ZT20-24 is only observed in WT, but not BKO. **A**. (top panel) Distribution of poke intervals of WT and BKO at ZT12-16. (bottom panel) Distribution of poke intervals of WT and BKO at ZT20-24. **B**. Proportion of premature (left), correct (middle), and late (right) trials. **: $p < 0.01$; *: $p < 0.05$ by Bonferroni's multiple comparison test following two-way ANOVA. Data are presented as mean with SD. n = 4 mice for WT, 3 mice for BKO. Data used for Figure 3 and 4 were re-analyzed for the plots.

and ZT20-24. During ZT12-16, we observed that the number of trials conducted in WT mice generally exceeded those in BKO mice, following the first poke and in early target durations (5-6 s) (Figure 5A, upper panel). In contrast, at ZT20-24, the distribution of the production interval exhibited a similar pattern in both WT and BKO mice, except the drop in the poke number in early target durations (Figure 5A, lower panel). Subsequently, we delved into an analysis of the proportion of premature, correct, and late trials at these two-time points (Figure 5B). Notably, the premature portion of BKO significantly increased compared to WT during ZT20-24 (Figure 5B, left panel), a period during which the production of the premature interval was at its nadir in the daily interval production patterns of WT mice. In contrast, at ZT12-16, the proportion of premature trials in WT and BKO mice did not exhibit a statistically significant difference. Interestingly, the pattern of correct trials was diametrically opposed to that of premature trials. A significant decrease in the proportion of correct trials was observed in BKO mice compared to WT mice during ZT20-24, while the proportion of correct trials did not significantly differ between WT and BKO mice during ZT12-16 (Figure 5B, middle panel). Notably, no significant patterns were discerned in the rate of late trials (Figure 5B, right panel).

Collectively, these results suggest that the interval timing capacity of BKO mice closely resembled that of WT mice during ZT12-16, regardless of the specific time points under examination.

Discussion

Our study presents evidence supporting the role of the molecular core clock in regulating second-level interval timing capacity, as assessed by a prospective motor interval production task. We observed a circadian pattern of interval timing in which wild-type mice demonstrated higher accuracy in interval production during the late part of the active phase (ZT20-24), while accuracy during the early active phase (ZT12-16) was compromised, characterized by an increased rate of premature intervals. While the genetic ablation of the *Bmal1* gene (BKO) did not disrupt the production of second-level intervals, the circadian pattern of interval production was not observed in BKO mice. Furthermore, the accuracy of interval production remained consistently lower in BKO mice due to the absence of the normal suppression of premature interval production seen at ZT20-24. In summary, we have identified that the optimal time of day for wild-type mice is the late part of the active phase when the suppression of prematurity is most pronounced. The results of BKO also suggest that core clock machinery regulates second-level interval production by timely suppressing premature interval production.

Despite the distinct mechanisms underlying the circadian clock and the second-to-minute timing system, they can interact with each other. The second-to-minute timing system is regulated at different times of the day in mice, rats, and humans (Shurtleff et al. 1990; Aschoff 1998; Agostino et al. 2011). Mutant mice with shorter (*Zfhx3*$^{Sci/+}$) or longer circadian free-running rhythms (*Afh* mutants) exhibit shorter and longer peak interval estimations, respectively (Balzani et al. 2016; Maggi et al. 2017). However, two independent studies have reported intact interval timing in core clock gene mutants, specifically *Clock*$^{-/-}$ or *Cry1*$^{-/-}$*Cry2*$^{-/-}$ mutants (Cordes and Gallistel 2008; Papachristos et al. 2011), which has sparked controversy. Notably, previous studies that reported intact interval timing in clock gene mutants tested the interval timing task only once a day (Cordes and Gallistel 2008; Papachristos et al. 2011). These observations suggest a model in which molecular circadian rhythms generate oscillations in the interval timing system. In this model, arrhythmic clock gene mutations may lock the interval timing system to a specific phase of the oscillation, resulting in a significant difference between the wild-type and mutant, particularly at a specific time of day. We employed an experimental design to examine the time-of-day effect on interval production behavior and demonstrated a significant genotype effect found only at ZT20-24 (Figure 5), supporting the proposed model.

What can be mechanisms of the circadian control of interval timing? We consider dopamine as a strong candidate. Dopamine regulates reward, motivation, motor output, social behaviors and emotions (Berke 2018; Wise 2004; Kim et al. 2022; Jung and Noh 2021). Additionally, it plays a pivotal role in interval timing. Lesions specific to dopamine neurons disrupt interval production behavior (Meck 2006), and manipulating dopamine receptors through pharmacological means affects performance in interval timing tasks (Drew et al. 2003; Drew et al. 2007). Furthermore, the administration of dopamine-related psychostimulants, such as amphetamine, modulates interval timing tasks (Taylor et al. 2007). Optogenetic and *in vivo* bulk calcium imaging studies have illuminated the causal role of midbrain dopaminergic neurons in temporal-categorizing tasks (Soares et al. 2016). The circadian rhythm of dopaminergic tone is governed through the transcriptional regulation of the dopamine biosynthesis pathway (Chung et al. 2014). In mice, dopamine levels peak at dawn during the transition from the active to resting phase and is the lowest during the transition from the resting to active phase (Kim et al. 2017). In this context, we report that the optimal performance in interval timing is observed at ZT20-24, which aligns with the peak phase of dopamine biosynthesis. This observation tempts us to speculate whether an increased dopaminergic tone during the latter part of the active phase causally enhances interval timing performance by suppressing premature interval production.

Second-level timing systems, the fundamental machinery for timekeeping in the brain, are associated with various cognitive functions, including foraging, associative learning, and decision making (Karson and Balcı 2021). Furthermore, interval timing perception is impaired in various neurological and psychiatric disorders, such as Parkinson's disease, substance misuse, attention deficit hyperactivity disorder, and schizophrenia (Pastor et al. 1992; Wittmann et al. 2007; Noreika et al. 2013; Snowden and Buhusi 2019). Therefore, investigating the circadian control of interval timing and its underlying mechanisms is essential to comprehend the role of the interval timing system in other cognitive functions and to establish interval timing tasks as diagnostic tools. In this study, we present evidence of a circadian pattern in motor interval production behavior and its dependence on core clock genes, contributing to a better understanding of the circadian control of interval timing. Further studies

employing circadian phase-specific manipulations and monitoring clock genes in a cell type-specific manner, such as in dopamine neurons, are warranted to gain a mechanistic understanding of the circadian control of interval perception.

Disclosure statement

No potential conflict of interest was reported by the authors).

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Code availability statement

The codes for operating and collecting data from the rig are available upon request.

Data deposition

The data presented in the study are available upon request.

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