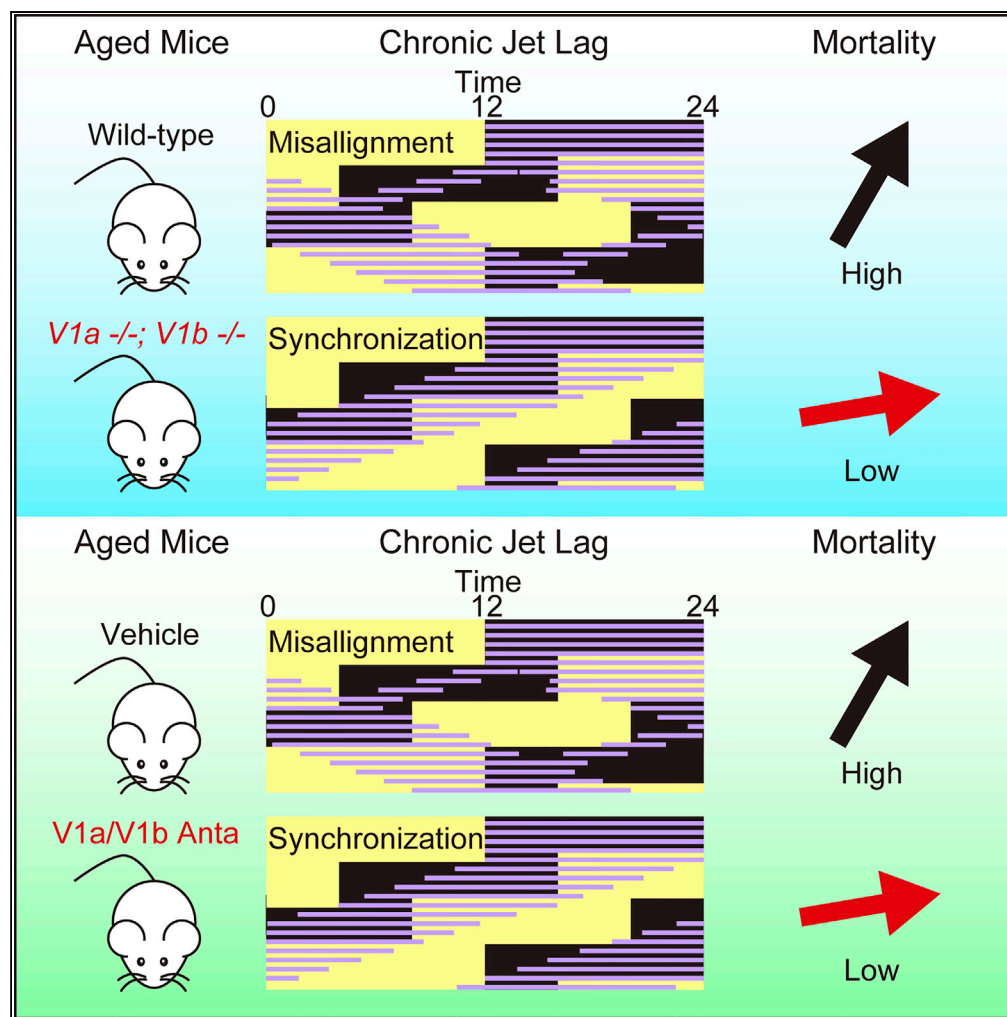


## Article

# Vasopressin Signal Inhibition in Aged Mice Decreases Mortality under Chronic Jet Lag



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## HIGHLIGHTS

Chronic jet lag increases mortality in aged mice

Rapidly resetting  $V1a^{-/-}; V1b^{-/-}$  mice showed lower mortality under chronic jet lag

Pharmacological inactivation of  $V1a/V1b$  signaling decreased mortality in aged WT mice

A potential pharmaceutical intervention for shiftwork-related health problems

Article

# Vasopressin Signal Inhibition in Aged Mice Decreases Mortality under Chronic Jet Lag

Yoshiaki Yamaguchi<sup>1</sup> and Hitoshi Okamura<sup>1,2,\*</sup>

## SUMMARY

**Chronic jet lag, a model of shiftwork, increases mortality in aged mice. One potential reason for this association is that the chronic desynchronization between the internal clock phase and the environmental light/dark (LD) cycle might increase the mortality rate. However, this hypothesis has not been examined because of the lack of an appropriate animal model to prove this speculation. Here, we found that rapidly entrainable vasopressin receptor  $V1a^{-/-}V1b^{-/-}$  mice showed lower mortality under a chronic jet lag condition. Moreover, we found that pharmacological inactivation of  $V1a$  and  $V1b$  signaling decreased mortality even in aged wild-type mice, thus providing a potential pharmaceutical intervention for shiftwork-related health problems.**

## INTRODUCTION

Approximately 20% of the working population in developed countries engage in some sort of shiftwork (Fritschi et al., 2011). Accumulating evidence reveals that shiftwork is a risk factor for cancers (Kubo et al., 2006; Schernhammer et al., 2001), obesity (Karlsson et al., 2001), diabetes (Pan et al., 2011), and heart diseases (Tenkanen et al., 1998). Studies using animals subjected to chronic jet lag (CJL) induced by shifting light-dark (LD) cycles repeatedly at regular intervals to mimic the environment of shift workers have reported that CJL is associated with rapid tumor progression (Filipski et al., 2004), obesity (Kettner et al., 2015), and heart diseases (Penev et al., 1998). Surprisingly, CJL was also shown to increase the mortality rate in aged mice (Davidson et al., 2006). However, the mechanisms underlying these correlations between shiftwork/CJL and deleterious health consequences are still unknown, even though the increase in the number of aged workers and their health problems remain unsettled.

Twenty-four-hour rhythms in physiology, metabolism, and behavior are generated by endogenous self-sustained circadian oscillators present in virtually all the cells in the body (Hastings et al., 2008; Silver and Kriegsfeld, 2014; Takahashi et al., 2008), which are governed by the master pacemaker located in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus (Mohawk et al., 2012; Moore and Eichler, 1972; Stephan and Zucker, 1972). Moreover, the SCN is the only site that receives the entraining signal from the environmental LD cycle (Mohawk et al., 2012); entrainment means that rhythmic behavioral or physiological events match their oscillation with that of an environmental cycle. Thus, the SCN is considered as the key site for entraining the circadian clock in a jet lag condition. One potential reason for the high risk of health problems in shift workers and experimental animals subjected to CJL, especially high mortality rate in aged mice, is the dissociation between the internal circadian rhythm (e.g., locomotor activity rhythms) and the external timing. In fact, compared with young mice, aged mice require more days to entrain to the new phase after an LD phase shift (Valentinuzzi et al., 1997). This strongly suggests that the circadian phase of behavior, which is controlled by the SCN, would be continuously desynchronized with the external time under CJL. However, the hypothesis that slow entrainment has a deleterious effect on health has not been examined because of the lack of an appropriate animal model to prove this speculation.

## RESULTS AND DISCUSSION

### Rapidly Entrainable $V1a^{-/-}V1b^{-/-}$ Mice Showed Lower Mortality under a Chronic Jet Lag Condition

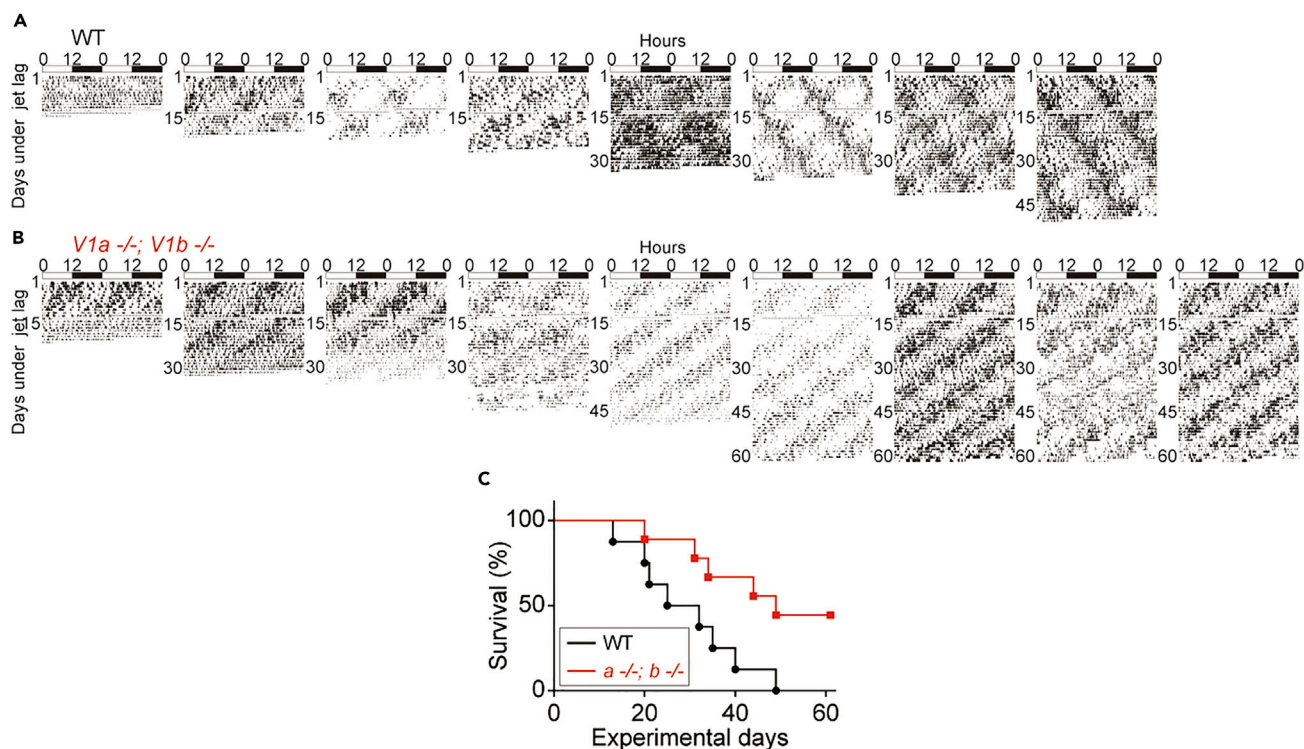
We previously found that mice deficient in vasopressin receptor  $V1a$  and  $V1b$  ( $V1a^{-/-}V1b^{-/-}$  mice) showed virtually no jet lag symptoms in behavior, clock gene expression, and body temperature rhythms after an LD

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**Figure 1. Survival Rate of Aged WT and  $V1a^{-/-}V1b^{-/-}$  Mice under a CJL Condition where LD Cycle Was Advanced by 8 hr Once Every 5 Days**

(A and B) Double-plotted actogram of all individual WT (A) and  $V1a^{-/-}V1b^{-/-}$  (B) mice under CJL. The actograms are plotted from the onset of chronic jet lag to the point of death. Top bars indicate initial LD cycle.

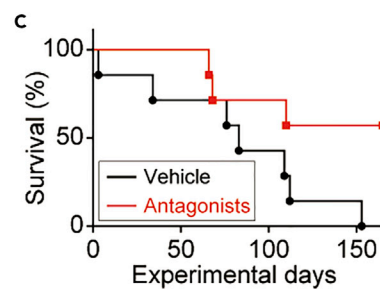
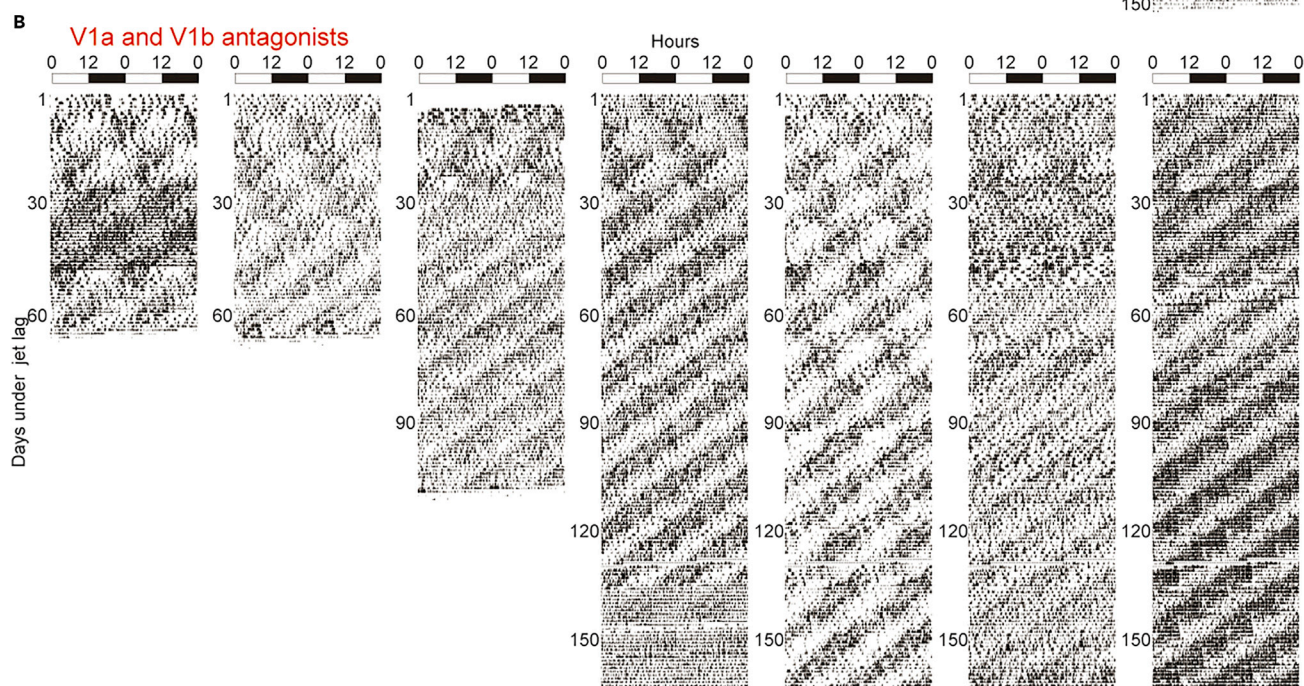
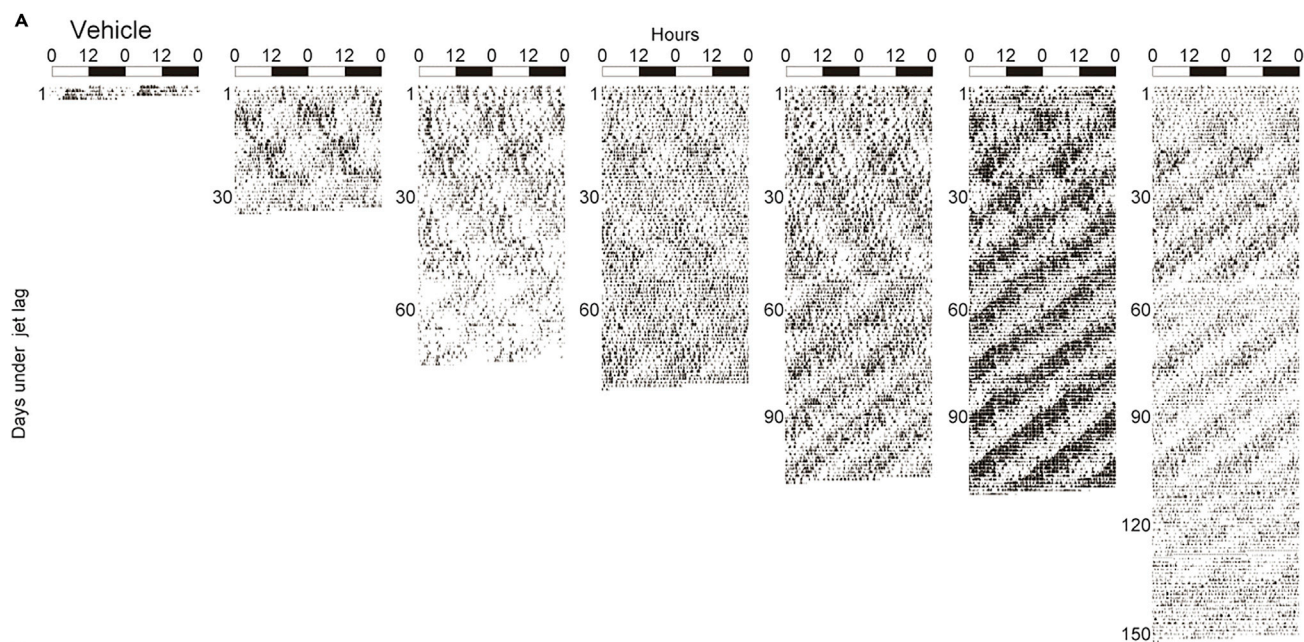
(C) Survival curves of aged WT and  $V1a^{-/-}V1b^{-/-}$  mice under CJL. Log rank (Mantel-Cox) test revealed a significant difference between the survival rates of WT and  $V1a^{-/-}V1b^{-/-}$  mice under CJL ( $n = 8$  for WT and 9 for  $V1a^{-/-}V1b^{-/-}$ ;  $p = 0.0191$ ). On day 61, the survival rate was 0% in WT mice and 44.4% in  $V1a^{-/-}V1b^{-/-}$  mice. Black and red colors indicate WT and  $V1a^{-/-}V1b^{-/-}$  mice, respectively.

See also Figure S1.

shift despite they having a normally functional clock (Yamaguchi et al., 2013). Moreover,  $V1a^{-/-}V1b^{-/-}$  mice developed normally and exhibited no gross abnormalities (Nakamura et al., 2009). Therefore, in this study, we aimed to use such rapidly entrainable  $V1a^{-/-}V1b^{-/-}$  mice and investigate whether the absence of dissociation between the internal clock and the environmental timing could overcome CJL-induced death in aged mice.

To induce CJL, i.e., a condition in which circadian oscillators will be recurrently forced to re-entrain to a new LD cycle, we placed 116-week-old wild-type (WT) and  $V1a^{-/-}V1b^{-/-}$  mice under CJL, where the LD cycle was advanced by 8 hr every 5 days. Until this age, 2 of the 10 WT mice and 1 of the 10  $V1a^{-/-}V1b^{-/-}$  mice died of natural causes, suggesting minimum difference between the longevity of WT and  $V1a^{-/-}V1b^{-/-}$  mice. We previously showed that circadian oscillations of clock genes not only in the SCN but also in the peripheral organs of  $V1a^{-/-}V1b^{-/-}$  mice fully re-entrained on day 5 after the 8-hr LD advance, whereas those in WT mice remained disturbed (Yamaguchi et al., 2013). We found that the locomotor activities in WT mice were not re-entrained continuously under CJL. WT mice even showed two locomotor rhythms with different period lengths simultaneously: one in the phase-advance direction and the other in the phase-delay direction (Figure 1A). Thus, WT mice showed a high locomotor activity in the light phase, which is not normal in nocturnal animals (Figure S1). WT mice steadily died after the onset of CJL, and all the mice examined died within 49 days under CJL. This mortality rate is quite similar with that observed in the previous study (Davidson et al., 2006). In contrast, the locomotor activities in  $V1a^{-/-}V1b^{-/-}$  mice quickly re-entrained after each LD advance and the mutant mice showed most of the locomotor activities in the dark phase (Figures 1B and S1). Approximately half the  $V1a^{-/-}V1b^{-/-}$  mice survived till day 61 after CJL initiation. Statistical analysis revealed that  $V1a^{-/-}V1b^{-/-}$  mice showed significantly less mortality rate than WT mice (Figure 1C).





**Figure 2. Survival Rate of Vehicle- or V1a/V1b Antagonists-Infused Aged WT Mice under a CJL Condition where LD Cycle Was Advanced by 8 hr Once Every 5 Days**

(A and B) Double-plotted actogram of all individual vehicle- (A) and antagonists-infused (B) mice under CJL. The actograms are plotted from the onset of chronic jet lag to the point of death. Top bars indicate initial LD cycle.

(C) Survival curves of aged vehicle- and antagonists-infused mice under CJL. Log rank (Mantel-Cox) test revealed a significant difference between the survival rates of vehicle- and antagonists-infused mice under CJL ( $n = 7$  each;  $p = 0.0461$ ). On day 165, the survival rate was 0% in vehicle-infused mice and 57.1% in antagonists-infused mice. Black and red colors indicate vehicle and antagonists treatments, respectively.

See also Figure S2.

**Infusion of V1a and V1b Antagonists into the SCN Decreased Mortality in Aged Wild-Type Mice**

Next, we examined whether inhibition of V1a and V1b signaling in the SCN is a key to decrease mortality in aged mice subjected to CJL. To approach this issue, we placed a cannula on the skull of WT mice and infused a mixture of V1a and V1b antagonists on the SCN continuously using a micro-osmotic pump (see [Transparent Methods](#) for the details). In this pharmacological study, we started the CJL exposure in mice aged 80 weeks to avoid potential sudden death due to surgical burden. Similar to the locomotor activities in aged WT mice examined above, vehicle-infused WT mice mostly showed a splitting behavior and considerably high locomotor activities in the light phase ([Figures 2A and S2](#)). All the vehicle-treated mice examined died in 153 days after CJL initiation ([Figures 2A and 2C](#)). In contrast, WT mice infused with a mixture of V1a and V1b antagonists showed a faster re-entrainment under CJL ([Figures 2B and S2](#)), and four of the seven mice examined survived till 165 days after CJL initiation ([Figures 2B and 2C](#)). Statistical analysis confirmed that the survival rate in antagonists-treated mice was higher than that of the vehicle-treated mice ([Figure 2C](#)).

The causal relationship between CJL and higher mortality rate in aged WT mice is still unclear. Chronic stress was probably not the main cause of this increased mortality since Davidson et al. reported that the total daily fecal corticosterone levels did not increase in aged WT mice subjected to chronic phase advances, which showed higher mortality (Davidson et al., 2006). However, our findings from experiments using  $V1a^{-/-}V1b^{-/-}$  non-jet-lag mice strongly suggest that long-term circadian misalignment between the endogenous circadian rhythm and the external LD cycle has an adverse effect on health in aged WT mice. In contrast,  $V1a^{-/-}V1b^{-/-}$  mice or V1a/V1b antagonists-infused mice showed less mortality rate under CJL. This higher survival rate could be attributed to the temporal alignment between the endogenous circadian rhythm and the environmental LD cycle via immediate resetting of the locomotor activity rhythm throughout the CJL period.

It was previously shown that aged C57BL/6 mice require longer days for re-entrainment after an LD advance compared with younger controls (Valentinuzzi et al., 1997). Moreover, cohort studies on nurses report that most nurses found it more difficult to cope with shift work as their age increases (Muecke, 2005). Although the precise mechanisms underlying the V1a- and V1b-mediated prevention of CJL-induced death remain unclear, the significant increase in survival rate of  $V1a^{-/-}V1b^{-/-}$  mice and V1a/V1b antagonists-infused mice may provide the initial steps for pharmaceutical intervention against shift work-related health concerns, which currently cannot be treated with any direct medication.

**METHODS**

All methods can be found in the accompanying [Transparent Methods supplemental file](#).

**DATA AND SOFTWARE AVAILABILITY**

The numbers of activity counts of each mouse have been deposited in the Mendeley Data repository (<https://doi.org/10.17632/xv4b7768xg.1>).

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Transparent Methods and two figures and can be found with this article online at <https://doi.org/10.1016/j.isci.2018.06.008>.

**ACKNOWLEDGMENTS**

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## AUTHOR CONTRIBUTIONS

Y.Y. and H.O. conceived and designed the study. Y.Y. conducted experiments. Y.Y. analyzed data. Y.Y. and H.O. wrote the paper.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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## **Supplemental Information**

### **Vasopressin Signal Inhibition in Aged Mice Decreases Mortality under Chronic Jet Lag**

**Yoshiaki Yamaguchi and Hitoshi Okamura**

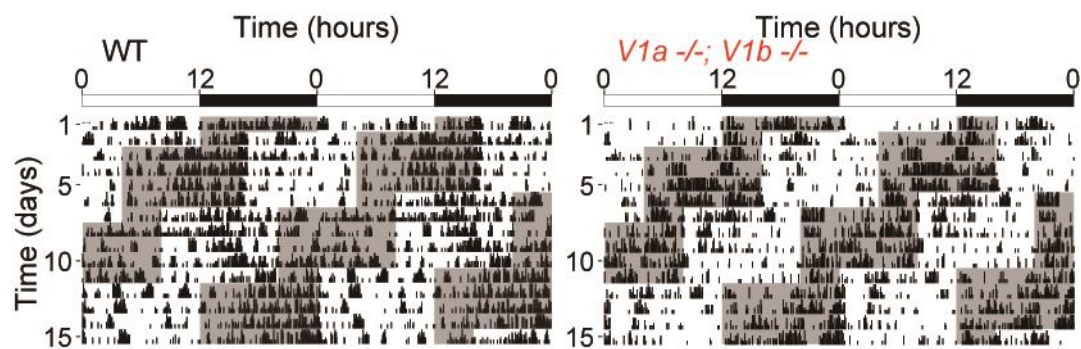


Figure S1. Related to Figure 1. Representative double-plotted actogram of WT (left) and *V1a*<sup>-/-</sup>*V1b*<sup>-/-</sup> (right) mice under CJL. White and gray background indicates lights on and off, respectively. Top bars indicate initial LD cycle.



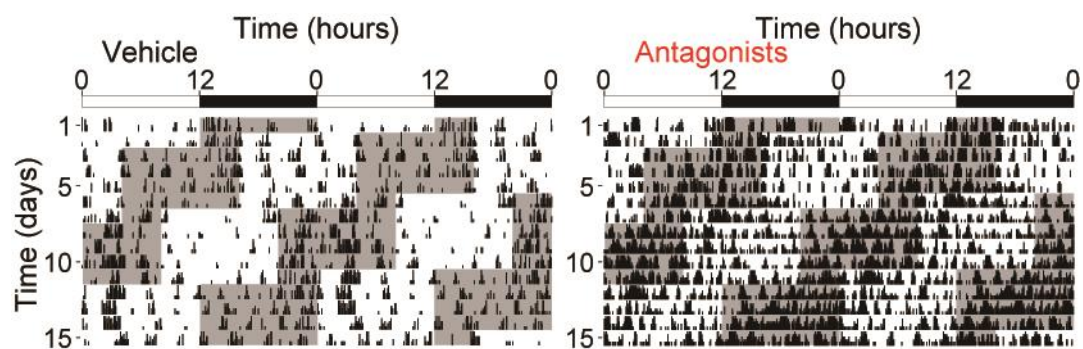


Figure S2. Related to Figure 2. Representative double-plotted actogram of vehicle- (left) and antagonists-infused (right) mice under CJL. White and gray background indicates lights on and off, respectively. Top bars indicate initial LD cycle.

## Transparent Methods

### Mouse and behavioral activity monitoring for jet lag experiments.

Wild-type mice (C57Bl/6 mice, male, 78-week-old) were purchased from Shimizu Laboratory Supplies (Kyoto, Japan). For comparing the mortality rate of WT mice and *V1a<sup>-/-</sup>V1b<sup>-/-</sup>* mice (Yamaguchi et al., 2013) under CJL condition, the mice were housed in the same facility from the age of 78 to 115 weeks. Then, each mouse was housed individually in light-tight, ventilated closets within a temperature- and humidity-controlled facility with *ad libitum* access to food and water. The animals were entrained on a 12-h-light (~200 lux fluorescent light)/12-h-dark cycle and the light-dark cycles were phase-advanced by 8-h once every 5 days. Locomotor activity was recorded in 5-min bins with a passive (pyroelectric) infrared sensor (FA-05 F5B; Omron), and the data obtained were analyzed using Clocklab software (Actimetrics) developed on MatLab (Mathworks). All the experiments were conducted in accordance with the ethical guidelines of the Kyoto University Animal Research Committee.

For pharmacological inhibition of V1a and V1b signaling, a mixture of OPC-21268 (2.5 mM, Sigma-Aldrich), a V1a antagonist, and SSR 149415 (2.5 mM, Axon Medchem), a V1b antagonist, was continuously delivered to the SCN via a micro-osmotic pump (Model 1004; ALZET). A hole was drilled at 0.5 mm posterior from the bregma. Then, the cannula (5 mm length, Brain Infusion Kit 2, ALZET) was inserted and fixed to the skulls of 79-week-old WT mice, and the pump was subcutaneously placed in the interscapular region. After the surgery, the animals were returned to their home cages and the LD cycles were advanced by 8-h once every 5 days. The pump was replaced with a new one containing the antagonist mixture or vehicle from ZT5 to ZT8 every 4 weeks under anesthesia (ZT stands for zeitgeber time; ZT0 indicates lights-on and ZT12 lights-off).

### Data and Software Availability

The numbers of activity counts of each mouse have been deposited in the Mendeley Data repository (<http://dx.doi.org/10.17632/xv4b7768xg.1>).