EIIRIS Electronics-Inspired Interdisciplinary Research Institute

概日リズムを外部コントロールし生活リズムを調節する研究

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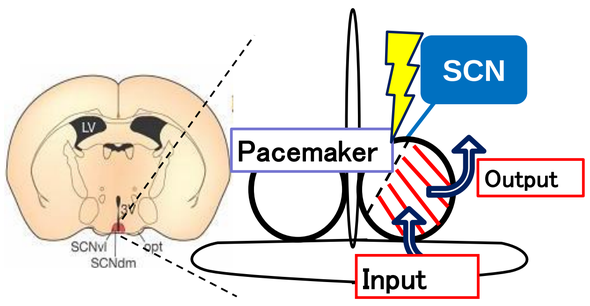
Introduction

Living things on the earth have internal clocks with about 24-hour period, which autonomously drive their physiology and behavior and reset their phase of circadian rhythms by environmental cues. Central circadian clock in mammals is located in the suprachiasmatic nuclei (SCN) in the hypothalamus. The SCN functions symmetry to the left-right axis of the body to keep normal circadian rhythms. However, what a mechanism to synchronize symmetry in the SCN has not been elucidated in detail.

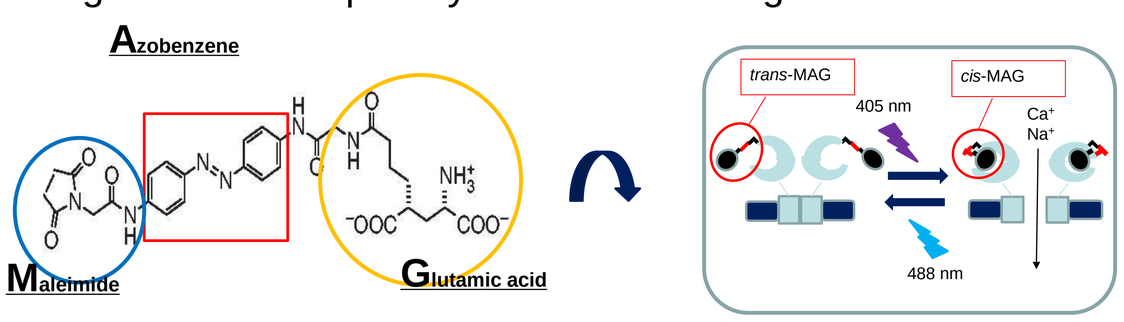


Material and Method

In this study, we observed the *Per1* expressional induction by photo-stimulation in *Per1* :: GFP transgenic (Tg) mouse. Only the left side of the SCN slice in Tg was photo-activated by a photo-reactive compounds NEO-MAG. GFP signal was compared between left and right part of the SCN slice, which was cut at the third ventricle of the median line to amputate contact of nerve fibers

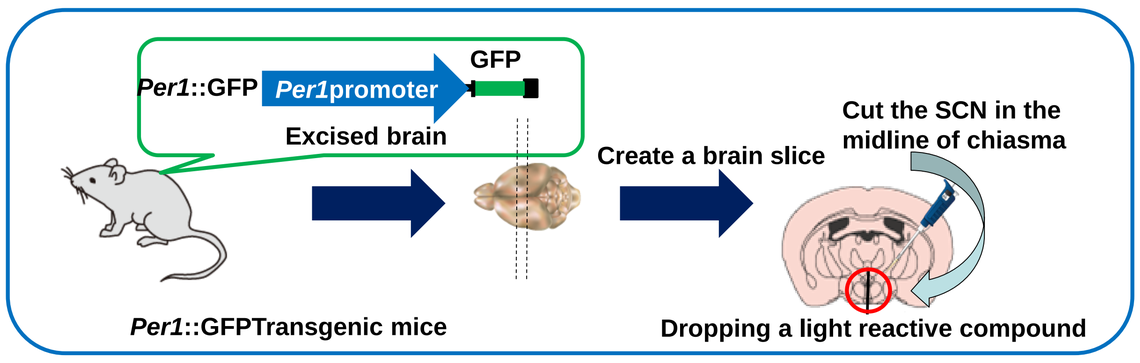
・Light stimulation system

The photoreactive compound MAG to operate ion channel of glutamate receptor by UV and visible light.



The NEO-MAG can phto-stimulate the local area of brain slice by laser irradiation in wildtype.

・Sample preparation



・Clock gene *Period1*(*Per1*) shows daily expressional rhythms in the SCN to regulate circadian rhythms. *Per1* expression is induced in the SCN about 30 to 90 minutes after the light stimulus to the eye to reset the phase of circadian rhythms.

<u>*Per1* induction could be monitored by GFP signal in *Per1*:GFP Tg.</u>

1 Left part of the SCN was photo-stimulated by a 405 nm and 488 nm laser, after adding NEO-MAG chemicals and cutting off the slice in ZT16.
2 Real-time observation of GFP signal in the SCN slice of *Per1* :GFP Tg mice was performed in once every 20 minutes for 8 hours.

・Light stimulus

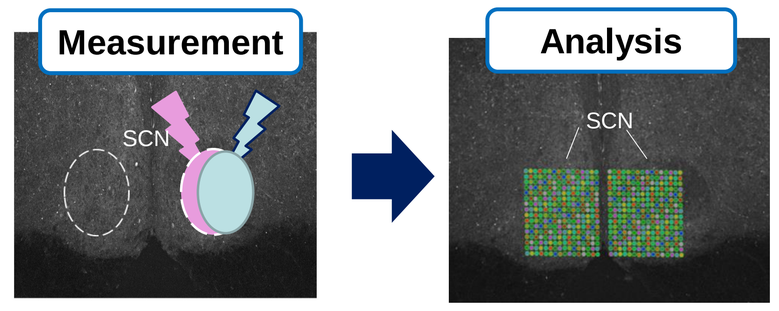
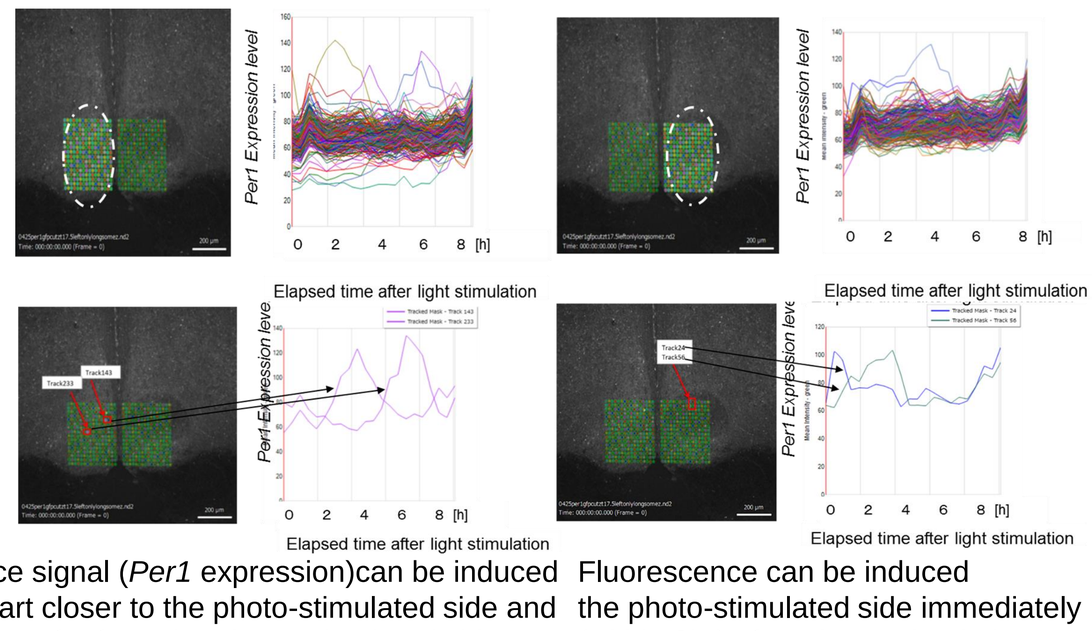


Photo-stimulation only on left side　　Measurement of the GFP signal

Result

Right (Non photo-stimulated) ⟺ Left (photo-stimulated)

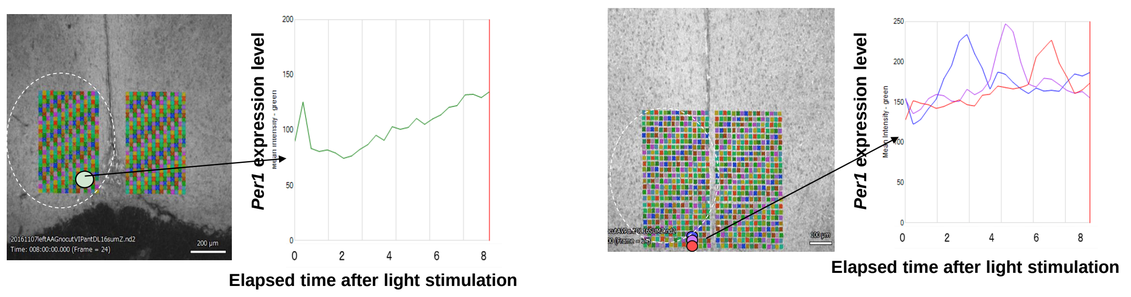


Fluorescence signal (*Per1* expression)can be induced fast in the part closer to the photo-stimulated side and slow in the part far from the photo-stimulated side

Fluorescence can be induced the photo-stimulated side immediately (within 3hr) after photo-stimulation.

GFP signal in right (Non photo-stimulated) after some kinds of inhibitor

Fluorescence signal can be induced also in the non photo-stimulated part (NPS) after adding AVP inhibitor in contrast to not after adding VIP inhibitor .



After adding VIP inhibitor　　After adding AVP inhibitor

Antagonist	chemical	f.c.	Effect in the NPS
VIP（VPAC2)receptor antagonist	Hederagenin	3μM	○
AVP（AVPV1a) receptor antagonist	SR49059	1 μM	×
GRP receptor antagonist	RD176252	0.4μM	×

The signals to reset the circadian rhythms is transmitted in the SCN for right-left functional synchronization by not AVP（AVPV1a) and GRP, but by VIP（VPAC2) and GABAa receptor .

Conclusion

1) Even after cutting the SCN slices in the midline of chiasma to block the contact of nerve fibers, the *Per1* expressional induction was also observed in the non-photostimulated side of the SCN as well as photo-stimulated side.
2) It is possible that not only by nerve contact but also by the humoral factors, the signals to reset the circadian rhythms is transmitted in the SCN for right-left functional synchronization by VIP and GABAa receptor.

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