Poster Session

The 14th Annual WPI-IIIS Symposium

～Science of Behaving and Sleeping Brains～

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1

Q Neurons Couple Sleep Architecture with Autonomic Regulation

Tohru M. Takahashi[1,2], Tomonobu Kato[3], Ai Miyasaka[12], Yoan Cherasse[1,2], Arisa Hirano[1,2], Takeshi Sakurai[1,2]

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Sleep and autonomic regulation are tightly coupled in mammals. During non-rapid eye movement sleep (NREMS), core body temperature decreases and sympathetic tone is suppressed, whereas during rapid eye movement sleep (REMS) thermoregulation is nearly abolished and autonomic activity becomes unstable. Yet, the neural substrates linking brain state and body physiology remain unclear.

We investigated QRFP-expressing neurons in the anterior median preoptic area of the hypothalamus—termed Q neurons—whose artificial activation induces hibernation-like hypothermia and bradycardia. To reveal their physiological function, we developed a chronic recording platform that simultaneously monitored Q-neuron GCaMP activity (Q-activity), EEG/EMG, core temperature, and heart rate in freely behaving mice.

During NREMS, Q-activity oscillated in an infraslow rhythm (~0.02 Hz, ~50 s) tightly coupled to sleep depth and cardiovascular fluctuations. These patterns were sufficiently predictive of sleep stage based on calcium signals alone. While Q-activity showed little overall correlation with body temperature, a positive relation emerged during transitions from prolonged wakefulness to NREMS. In contrast, Q-activity declined just before REMS onset and was completely silenced during sustained REMS. Within NREMS, transient "surges" of Q-activity were frequently associated with microarousals, reductions in δ (1.5–4 Hz) and σ (10–15 Hz) EEG power, and elevations in heart rate and EMG—dynamics resembling those of locus coeruleus noradrenergic neurons.

These findings identify Q neurons as a previously unrecognized preoptic node coupling autonomic control to sleep architecture, functioning more like arousal-modulating neurons than classical thermoregulatory cells, and providing a mechanistic link between brain state and systemic homeostasis.

2

Function of dopamine receptor D2-expressing neurons in the central nucleus of amygdala in sleep-wake regulation

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The amygdala is the brain's central hub for emotional processing and is implicated in sleep dysregulations such as stress-induced insomnia and nightmares. However, the specific mechanisms by which the amygdala regulates sleep are still not fully understood. Although previous study reported that optogenetic inhibition of dopamine receptor D2 (Drd2)-expressing neurons (Drd2 neurons) in the basolateral amygdala triggered NREM-to-REM transition and increased total REM sleep time, the role of Drd2 neurons in central nucleus of the amygdala (CeA) remains unknown.

Our current study aims to investigate the role of Drd2 neurons in the CeA in sleep and wakefulness regulation. Using anterograde tracing, we found that Drd2 neurons in CeA project to regions known to promote wakefulness and regulate REM sleep. Then, we identified the neuronal activity of Drd2 neurons in the CeA across different sleep-wake stages by combining fiber photometry and sleep recording. We observed that the calcium-dependent fluorescence levels from GCaMP6 increased during the transition from NREM to REM sleep and decreased during the transition from REM to wakefulness. We also manipulated Drd2 neurons in the CeA using optogenetics during each sleep stage and observed that artificial stimulation of these neurons leads to immediate wakefulness while inhibition during NREM sleep leads to increased latency to REM sleep without affecting total sleep time. Lastly, we hypothesized that optogenetic manipulation of Drd2 neurons in the CeA during REM sleep might influence emotional memory consolidation. Together, our findings uncover a new role for CeA Drd2 neurons in regulating sleep and wake, suggesting potential new therapeutic targets for sleep-related disorders.

3

Artificial hibernation: Cortical dynamics and Neural Substrates of QIH state

Rafaël Michaud[1], Shingo Soya[1,2], Takeshi Sakurai[1,2]

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Recently, an artificially induced hibernation-like state,

termed QIH (Q neuron–induced hypothermia and hypometabolism), has been described in mice. The underlying neural mechanisms and their impact on brain activity remain poorly understood. Here, we provide a detailed characterization of cortical dynamics during QIH, identify its neuronal substrates, and quantify its influence on neurophysiological processes. We combined Neuropixels recordings with optogenetic activation of Q neurons in freely moving mice, analyzing local field potential (LFP) and single-unit activity.

We identified two distinct cortical states: an inactive QIH state, linked to behavioral quiescence, showed light delta power (0.5–4.5 Hz) and loss of neuronal synchrony; an active QIH state, associated with residual activity, displayed a beta/gamma band (25–45 Hz) distinct from normal gamma (45–65 Hz), reflecting slowed oscillations and decreased fast-spiking neuron depolarization rates. The inactive-to-active ratio was higher during the light period and overall higher than in normal states. These spectral changes were absent during warm-QIH stimulation, which prevented hypothermia, although REM episodes were suppressed. Post-QIH, sleep/wake balance was altered, with increased REM and wake periods alongside enhanced slow-wave activity during NREM sleep.

These findings indicate that QIH does not simply shut down cortical activity but comprises two circadian-regulated states. While Q neuron stimulation indirectly modulates cortical signatures of QIH via hypothermia, it directly suppresses REM. Ongoing work examines PV+ neurons as potential hypothermia-sensitive targets underlying spectral changes, and the role of recently discovered hypothalamic wake/NREM/REM populations in mediating REM inhibitio.

4

Development of novel small-molecule bradykinin 2 receptor agonists via structure-based antagonist-to-agonist switching

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Bradykinin 2 receptor (B_2R) is a G protein-coupled receptor (GPCR) involved in various physiological functions, including vasodilation and increased vascular permeability. Notably, intracerebral administration of bradykinin induces prolonged sedation and exerts anti-aversive effect, indicating its potential relevance in central nervous system. B_2R agonists are expected to have therapeutic potential as treatments for ischemic diseases, cancer treatment, and neuropsychiatric disorders like panic disorders. However, existing B_2R agonist are limited by poor solubility and low brain permeability due to their high molecular weight.

Recently, we discovered that the introduction of a 2-pyridylmethyl group on the quinoline core of B_2R antagonist, fasitibant, successfully converted its activity from antagonism to agonism, yielding a novel B_2R partial agonist, FaD. In this study, we aimed to develop lower molecular weight B_2R agonists by derivatizing novel derivatives through rational derivatization based on cryo-electron microscopy structures of the fasitibant– and FaD–B_2R complexes.

Structural analyses of the ligand–B_2R complexes revealed that interactions involving the ornithine and piperazine moieties are not essential for the induction of agonist activity. Based on these insights, we designed and synthesized derivatives lacking these substructures. Remarkably, these derivatives retained partial agonist activity at B_2R, with approximately tenfold lower potency compared to FaD. These findings underscore the feasibility of structural simplification and offer a promising direction for the development of low-molecular-weight B_2R agonists with potential applications in treating central nervous system disorders. In this presentation, we will discuss the design strategy, synthetic methods, and detailed pharmacological evaluation of these novel B_2R agonists.

5

Efficient synthesis of novel DOI derivatives for the serotonin 2A receptor.

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Depression and depressive states are often accompanied by various sleep disturbances, including insomnia. Conventional antidepressants suffer from limitations such as delayed onset of action and poor efficacy in treatment-resistant depression (TRD). Recently, activation of the serotonin 2A receptor ($5\text{-}HT_{2A}R$) has been suggested to exert rapid and robust antidepressant effects, even in TRD. However, $5\text{-}HT_{2A}R$ activation is also associated with hallucinogenic effects, posing a major therapeutic challenge. Interestingly, 2,5-

dimethoxy-4-iodoamphetamine (DOI), a 5-HT_{2A}R agonist, has been reported to produce antidepressant effects without inducing hallucinations at therapeutic doses. In this study, we aimed to develop a synthetic route for DOI derivatives that retain antidepressant activity without hallucinogenic effects. A late-stage derivatization strategy was designed to enable efficient functional modification of DOI. To achieve selective deprotection of both the 2,5-dimethoxy moieties and the amino substituent, methoxymethyl (MOM) and phthalimide protecting groups were introduced, respectively, allowing the synthesis of key intermediates. Selective deprotection of these intermediates enabled the preparation of six derivatives modified at the methoxy positions and several derivatives at the nitrogen atom. Pharmacological evaluations were performed for these compounds. Among them, 5-hydroxy-DOI (5-OH DOI) exhibited 5-HT_{2A}R activation equivalent to that of DOI, but its hallucinogenic activity was reduced to one-third. In this presentation, we will describe the synthesis and pharmacological characterization of these DOI derivatives in detail.

6

Development of novel *in vivo*-applicable SIK3 selective activators

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Salt-inducible kinase 3 (SIK3) has been reported to play a key role in the regulation of sleep, and to elucidate its detailed mechanism, chemical tools to specifically activate SIK3 *in vivo* are required.[1,2] Recently, Yanagisawa et al. found that artepillin C was able to activate SIK3. However, artepillin C shows poor stability toward heat and light, poor water solubility, and limited SIK3 selectivity, which make its *in vivo* application difficult. To overcome these issues, we designed and synthesized a series of derivatives based on the structure of artepillin C to investigate their structure–activity relationships (SARs).
First, we established an efficient method for introducing a prenyl group, which is important for SIK3 activation, and successfully synthesized artepillin C and its analogs. Using this method, we also prepared a versatile common intermediate that enabled efficient access to structurally diverse derivatives. From this intermediate, we synthesized compounds bearing various functional groups. Secondly, we synthesized compound YKK-342, YKK-345, and YKK-366, which have distinct scaffolds, to investigate the active conformation of artepillin C.
In this presentation, we will describe the synthetic strategy for these novel derivatives and discuss their SIK3 activation profiles in relation to their structural features.

【references】

1) M. Yanagisawa *et al*. *Nature*, **2016**, *539*, 378.
2) H. Funato *et al*. *Nature*, **2022**, *612*, 512.

7

Targeting the delta opioid receptor for novel therapeutics in stress-related disorders

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Stress-related disorders impact more than one billion people worldwide and are now recognized as a major global social issue. However, patient satisfaction with current treatments remains low, and increasing healthcare costs and declining labor productivity heavily burden the global economy. These challenges highlight the critical necessity for developing new therapeutic approaches.
In our research, we have focused on the delta opioid receptor (DOP)—a subtype of the opioid receptor family that is abundantly expressed in brain regions associated with emotional regulation—and have progressed in drug discovery efforts targeting this molecule. Through a series of experiments conducted in rodent models, it has been demonstrated that DOP agonists may exert therapeutic effects across multiple stress-related disorders, including depression, anxiety disorders, post-traumatic stress disorder (PTSD), and irritable bowel syndrome (IBS). Currently, clinical trials are underway to assess novel DOP agonists as potential antidepressants. More recently, we have also shown that DOP activation produces robust anti-stress effects in response to emotional stress paradigms.
These findings strongly suggest that DOP is a promising target for both preventing and treating stress-related disorders. Additionally, research focused on DOP is expected to improve understanding of the

pathophysiology and disease mechanisms behind stress-induced conditions. In this presentation, we will introduce our recent findings on the pharmacological actions of DOP agonists and discuss the underlying mechanisms.

8

Novel antiseizure intervention based on positive allosteric modulators of adenosine A2A receptors

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Epilepsy is the fourth most prevalent neurological disorder globally, affecting approximately 50 million individuals. It is marked by recurrent, unprovoked seizures that vary in type and severity. Our previous research has established a key function of adenosine A_{2A} receptors (A_{2A}Rs) in sleep regulation within the nucleus accumbens, and we have developed a positive allosteric modulator (PAM) of A_{2A}Rs—A_{2A}RPAM-1—for treating insomnia. Here, we explored the potential of A_{2A}RPAM-1 in modulating epileptic activity. Using pentylenetetrazole (PTZ), which is commonly employed in epilepsy models, we examined seizure outcomes with and without A_{2A}RPAM-1 pretreatment. We combined video analysis of seizure behavior with EEG/EMG recordings, focusing on the first hour post-injection. A_{2A}RPAM-1 pretreatment significantly shortened PTZ-induced seizure duration and reduced the number of seizure spikes observed in EEG recordings. Seizure episodes were categorized by duration: short (4 seconds), medium (4–12 seconds), and long (>12 seconds). Mice pretreated with A_{2A}RPAM-1 showed a marked reduction in short and total seizure episodes. We also assessed postictal depressive episodes (PDEs), which typically follow generalized tonic-clonic seizures (GTCS) and are characterized by sustained high-amplitude EEG waves. A_{2A}RPAM-1 pretreatment dramatically reduced the incidence of both PDEs and GTCS. These findings suggest that modulation of A_{2A}Rs may offer a novel intervention for severe epileptic seizures.

9

Dopaminergic signaling in the nucleus accumbens mediates affective reactivity following acute vicarious social defeat stress

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Dysregulated affective reactivity contributes to hyperarousal, a core feature of insomnia, but the neural circuits mediating this link remain unclear. This study investigates how acute vicarious social defeat stress (VSoD), a paradigm in which a bystander mouse witnesses a conspecific being defeated, affects behavioural and neural activity in mice. Electroencephalogram and electromyogram recordings were used to assess sleep–wake states following VSoD exposure, alongside post hoc video analyses to quantify emotional stress-related behaviours. The most relevant behaviour identified was defined as partition approaching (PA), a marker of affective reactivity. PA occurred both during the VSoD session and within one hour afterwards, coinciding with an increase in total wakefulness compared to control conditions. Immunohistochemical analysis of c-Fos expression revealed increased neural activation in the nucleus accumbens (NAc) and ventral tegmental area (VTA) associated with PA behaviour. Dopamine activity in the NAc increased during and after VSoD, but decreased when dopamine afferents, particularly those from the VTA were inhibited. To examine the role of dopamine signalling, chemogenetic inhibition of D1R- and D2R-expressing neurons in the NAc was performed. Inhibition of D1R neurons significantly reduced PA behaviour, whereas inhibition of D2R neurons had a lesser effect. These findings suggest that dopaminergic activity within the NAc modulates affective reactivity following VSoD in mice.

10

Mechanisms of Slow Wave Generation via the Histamine System

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Slow waves in electroencephalograms (EEGs) are a

hallmark of sleepiness and are typically observed during non-rapid eye movement (NREM) sleep and drowsy wakefulness. Antihistamines, widely used to treat allergic conditions, act as inverse agonists of the histamine H_1 receptor (H_1R) and are well known to induce drowsiness. In rodents, it has been reported that antihistamines increase NREM sleep duration and enhance delta power in EEG recordings. However, the neuronal mechanisms underlying H_1R-mediated drowsiness remain poorly understood.

In this study, we found that the enhancement of slow-wave activity induced by antihistamine during NREM sleep was attenuated by gabapentin, a pharmacological blocker of thrombospondin-1 (TSP1) receptors (α2δ-1 subunits). TSP1, secreted from astrocytes, promotes synapse formation, suggesting that astrocytic signaling contributes to H_1R-mediated slow-wave generation. These findings provide new insights into the cellular basis of slow-wave activity and the mechanisms that drive sleepiness.

11

A novel molecular integrator of sleep need in the ventral striatum

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The molecular mechanisms underlying progressive accumulation of sleep need during wakefulness, remain poorly understood, and no definitive molecular marker has been identified. The ventral striatum, a dopamine-rich hub is a key but underexplored center for regulating sleep–wake states and the molecular encoding of sleep pressure. To address this gap, transcriptomic profiling of ventral striatum was performed across different sleep conditions (sleep, sleep deprivation, and Sik3 mutant), leading to the identification of a previously uncharacterized gene, provisionally named NIND (from the Hindi word for “sleep”), which emerged as a robust molecular marker of sleep homeostasis. Quantitative PCR confirmed elevated NIND expression in the ventral striatum, with peak levels observed at the end of the wake phase in wild-type mice. Protein–protein interaction analysis using the STRING database identified NIND in association with 14-3-3-zeta, suggesting its integration into established sleep homeostasis networks such as the Sik3 signaling pathway. Furthermore, NIND expression was upregulated across D1-, D2-, and D3-type striatal neurons following acute sleep deprivation (SD). Future studies employing cell type–specific CRISPR/Cas9-mediated knockdown (KD) of NIND in striatal neurons, combined with EEG/EMG monitoring after SD, will help elucidate its role in regulating sleep pressure. A pilot study in D1Cre/Cas9-positive mice with NIND KD revealed a reduction in slow-wave sleep duration accompanied by increased wakefulness, without notable alterations in REM sleep. Following acute SD, these mice exhibited a trend toward reduced recovery sleep compared to controls. Collectively, these findings suggest that loss of NIND in D1-expressing neurons in the ventral striatum disrupts sleep homeostasis, possibly though enhanced neuronal excitability. However, the underlying mechanisms remain to be elucidated. These findings identify NIND as a potential molecular integrator of sleep need in the ventral striatum, laying a foundation for future investigations into the molecular mechanisms underlying sleep homeostasis and hyperarousal disorders.

12

Elucidating the Impact of Targeted Memory Reactivation on Fear Memory in Mice

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Targeted Memory Reactivation (TMR) is a technique that modulates specific memories during sleep by presenting external cues, such as sounds. Studies have reported that TMR applied during non-rapid eye movement (NREM) sleep reduces fear responses in both rodents and humans, suggesting its therapeutic potential for post-traumatic stress disorder (PTSD). However, the optimal micro-architectural features and precise timing within the sleep–wake cycle that determine the efficacy of TMR remain largely unknown.

This study aims to elucidate the effects of sleep stage-specific TMR on the consolidation and modification of fear memories in mice, thereby contributing to the development of novel therapeutic strategies for PTSD. Using a classical fear-conditioning paradigm, we apply auditory TMR during post-conditioning NREM sleep and focus on how timing-dependent NREM sleep features influence memory consolidation and modification.

We have developed an experimental platform utilizing an AI system that accurately identifies sleep stages in

mice in near real-time by analyzing EEG signals. This system enables the automatic delivery of auditory cues timed to specific sleep stages, allowing for a systematic investigation into how subtle timing differences during NREM sleep influence the efficacy of TMR in fear memory.
We are beginning to identify TMR timing-specific NREM sleep features that influence the effects of NREM-targeted TMR on fear memory. In this presentation, we will report our latest insights.

13

REM sleep rhythm impairment in people with PTSD

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Over 90% of patients with post-traumatic stress disorder (PTSD), a psychiatric disorder that develops after a life-threatening event, report sleep maintenance difficulties (Neylan et al., 1998). However, objective evaluations of sleep variables do not always reflect the sleep disturbances (Kobayashi et al., 2007; Zhang et al., 2019; Baglioni et al., 2016). One possible reason is that unfamiliar setting of traditional polysomnography (PSG) which require hospitalization and multiple sensors and may alter natural sleep architectures (Zhang et al., 2019). To capture habitual sleep, we conducted multi-night in-home sleep recordings using a portable PSG device, InSomnograf (Masaki et al., 2025).
The results showed that PTSD patients had a total sleep time similar to that of healthy individuals but exhibited lower sleep efficiency and unregular sleep habits. This suggests that subjective sleep disturbances in PTSD may be more related to sleep quality than quantity. Additionally, significant REM sleep abnormalities were identified, including reduced total duration per night, prolonged latency, and increased fragmentation. Moreover, analysis of the temporal dynamics of the occurrence probability of each sleep stage revealed a disruption in REM sleep cyclic pattern and subsequently in slow wave sleep. From this novel rhythmicity analysis, the rhythmic occurrence of REM sleep may shape sleep cycles and play a key role in sleep quality.
Furthermore, since REM sleep is crucial for processing emotional memories including fear memory extinction, REM sleep dysfunction which we found may provide insights not only into symptomatic treatment of sleep disturbances but also into more fundamental PTSD treatment approaches.

14

Unraveling the Metabolic Functions of Postprandial Sleep via Brain–Viscera Interaction

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Postprandial sleep (PPS) is an evolutionarily conserved form of sleep that occurs after feeding across a wide range of species. PPS has been considered to conserve energy for digestion. However, the actual amount of energy saved appears to be quite limited. In addition, PPS has been implicated in the secretion of satiety-related hormones, the acquisition of starvation tolerance, and the regulation of metabolic functions. Recent studies have revealed that the gut–brain axis regulates the functions of peripheral organs. It is therefore conceivable that neural signals originating from the brain during PPS may influence digestive, absorptive, and metabolic functions in peripheral organs. However, our understanding of the physiological roles of PPS remains limited, and its functional significance is still largely unknown.
This study aims to elucidate the physiological role of PPS as a state that optimizes metabolic functions through brain–viscera interactions, rather than merely representing rest during digestion.
Using a mouse model we established to induce PPS during the active (dark) phase with a high-nutrition diet, we examined gene expression changes in peripheral organs following PPS. RNA-seq analysis of the small intestine comparing PPS-induced mice with those subjected to sleep deprivation after PPS induction revealed significant activation of steroid hormone synthesis and retinol metabolism pathways.

Furthermore, Gene Ontology (GO) analysis indicated increased enrichment of terms related to lipid metabolism, iron and protein absorption, and neurotransmission. These findings suggest that PPS enhances digestive, absorptive, and metabolic functions in the small intestine.

15

Cell Surface and Intracellular Dynamics of mGluR5 in Response to Sleep Pressure and Sleep Regulation Mechanisms

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Sleep deprivation is a prevalent public health issue, yet the fundamental cellular mechanisms for tracking sleep pressure remain unknown. The metabotropic glutamate receptor 5 (mGluR5), a postsynaptic receptor highly expressed in the cortex, is a key candidate. Human PET studies show increased cell-surface mGluR5 in cortical areas (M1, V1) during sleep deprivation; however, PET lacks the cellular resolution to define these trafficking dynamics. We hypothesized that mGluR5 traffics to the surface during periods of high sleep pressure and is internalized during periods of low sleep pressure. To test this, we used mGluR5-SNAPtag mice to visualize receptor localization (using membrane-impermeable /permeable ligands) and performed GCaMP6f calcium imaging in wild-type brain slices to determine functional consequences.

Calcium imaging revealed that after sleep deprivation, both the M1 and V1 cortices showed significantly lower signal peaks following whole-cell stimulation. However, M1, but not V1, exhibited a significantly prolonged signal duration, suggesting M1 is uniquely sensitive. Critically, selective activation of surface-only mGluR5 mimicked this prolonged signal kinetics, supporting our hypothesis that sleep deprivation promotes surface trafficking. Additionally, our SNAPtag imaging in hippocampal cultures confirmed the surface localization of mGluR5 to dendritic spines. It also demonstrated that surface agonist application significantly increases the surface-to-total mGluR5 ratio, indicating activity-dependent trafficking of this receptor. A detailed analysis of receptor trafficking in acute brain slices is currently underway. Future studies will focus on validating these results in vivo to define the role of mGluR5 as a key sensor and regulator of homeostatic sleep pressure.

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Brain-wide identification of neurons activated by sleep deprivation using TRAP2 mice

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Sleep deprivation (SD), achieved by elevating arousal through external stimulation, causes the accumulation of sleep pressure. As SD continues, the brain enters a competitive state between sleep drive and externally induced arousal. Consequently, both sleep- and wake-promoting neurons may be simultaneously active, although the underlying brain state remains poorly understood.

We used TRAP2;hM3Dq double transgenic mice to label neurons activated by 6 h of SD and to assess their function through chemogenetic manipulation. The TRAP2 system (Targeted Recombination in Active Populations 2) employs an activity-dependent *Fos* promoter to drive Cre recombination in the presence of 4-hydroxytamoxifen, leading to permanent expression of hM3Dq–mCherry. Clozapine-N-oxide (CNO) administration selectively reactivated these neurons, allowing us to monitor resulting changes in EEG activity and body temperature.

Unexpectedly, CNO-induced reactivation decreased body temperature in both control and SD groups. In the SD group, two distinct phenotypes emerged: one subset exhibited enhanced EEG activation and rapid recovery from hypothermia, whereas another showed global EEG suppression and prolonged hypothermia. These opposing effects may reflect differences in the proportions of wake- versus sleep-promoting neurons labeled by TRAP2. Comparative histological analyses will be used to identify and distinguish these neuronal populations.

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Nemuri-resistant bacteria as a tool to explore putative new regulators of sleep

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Nemuri was identified in *Drosophila* as a peptide with

both anti-microbial and sleep-inducing activity. Recently, we revealed that Nemuri-derived disordered peptides accumulate in bacterial ribosome-rich cytoplasmic condensates— formed through liquid–liquid phase separation (LLPS)— to reduce ribosomal fluidity, alter protein translation, and mediate an anti-microbial activity. Yet, how Nemuri operates in *Drosophila* brain neurons to induce sleep is still to be investigated.
An emerging Nemuri-resistant strain of bacteria will harbor molecular networks that would reverse the Nemuri anti-microbial effect mediated by hardening of LLPS condensates and the associated changes in ribosomal and/or mRNA dynamics. We anticipate that similar or relevant Nemuri-interacting molecular networks may also operate in *Drosophila* brain neurons to regulate Nemuri-induced sleep.
We adapted a work protocol to grow ATCC25922 strain of *E.coli* at an optimized sub-lethal concentration of a Nemuri-derived peptide. This creates selective pressure through cycles of serial transfers at regular intervals in order to find emerging isolates that can survive at previously lethal peptide concentrations. Mutation mapping using unbiased whole-genome sequencing to compare the emerging resistant isolates with the ancestor strain can reveal resistance-conferring mutations and the relevant molecular networks. Mutation reconstruction screens in the ancestor bacterial strain can identify molecular candidates that are directly involved in conferring resistance. Based on this procedure, transgenic fly lines or genomic editing can be leveraged to test how Nemuri-induced sleep is modulated by these molecular manipulations.

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Identification of brainstem circuits crucial for REM sleep

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Understanding the circuitry underlying rapid eye movement (REM) sleep has been hindered by the remarkable neuronal diversity within the brainstem. In this study, we demonstrate in mice that a population of neurons in the pontine sublaterodorsal tegmentum (SubLDT) expressing corticotropin-releasing hormone-binding protein (*Crhbp*) and projecting to the medulla promotes REM sleep. Selective ablation of these *Crhbp* neurons reduces REM sleep and impairs REM sleep atonia. In the medullary region receiving inputs from *Crhbp* neurons, we identified nitric oxide synthase 1–expressing (*Nos1*) neurons that form a reciprocal projections with the SubLDT and also promote REM sleep, suggesting a positive feedback loop between the pons and medulla that forms a key component of the REM sleep circuitry. Moreover, *Nos1* neurons project to forebrain regions that regulate global brain activity, suggesting their involvement in forebrain activation during REM sleep.

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Neural mechanisms underlying the rapid induction of a pup calm state by mother–pup interaction in mice

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Newborn humans and mice cannot move freely by themselves, and are often carried by their parents. During transport, infants show a calming response, in which they stop moving, stop crying, and their heart rate decreases. In mice, pups exhibit a characteristic posture known as the transport response (TR) when carried by grasping their posterior neck, which facilitates maternal transport. This behavior shares several features with sleep, such as immobility, relaxation, and rapid reversibility, although its underlying mechanisms remain poorly understood. Here, we investigated features of TR focusing on changes in the electroencephalogram (EEG) and the factors that are involved. We revealed that EEG during TR resemble those during deep NREM sleep and that certain neuromodulators are involved in its regulation.

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Understanding the link between cellular homeostasis and neuronal slow wave activity through synaptic plasticity.

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Current evidence suggests that the regulation of cortical neurons at the cellular level, including factors such as synapse potentiation, DNA damage, and oxidative stress, affects sleep. In this study, we used a mathematical model, bioinformatics, and a multi-electrode array (MEA) to investigate whether these cellular-level factors play a role in sleep regulation and homeostasis.
Initially, we investigated whether synaptic potentiation triggers sleep using a two-population mathematical model composed of excitatory and inhibitory neuronal populations. Our model demonstrates that synaptic potentiation among excitatory neurons can facilitate the transition from awake-like firing to sleep-like firing patterns.
To validate the predictions from the mathematical model, we utilized an MEA system. The administration of a positive allosteric modulator of AMPA receptor to dissociated cortical neurons on MEA induces synchronized sleep-like firing patterns and increase delta power in the local field potential.
We then investigated public bulk and single-cell RNA-seq datasets acquired under sleep deprivation (SD). We found that most of SD-associated transcriptional modules, are related to processes that maintain homeostasis. From this observation, we hypothesize that homeostatic imbalance influences synaptic strength of cortical neuron. By utilizing in vitro system that mimics prolonged wakefulness, including electrical activity pattern, metabolism, and spine dynamics, we linked SD-related modules to long-term potentiation pathways and sleep-regulatory kinases.
Together, our findings suggest that neural activity during wakefulness imposes a cost to cellular homeostasis, a fundamental trade-off for neurons, and that cortical synaptic strength may encode the level of intracellular imbalance as a proxy for sleep need.

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Development of a Machine Learning Model to Estimate Psychomotor Vigilance Test Response Speed from Eyelid Opening Degree

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Responsiveness to stimuli declines under conditions such as sleepiness, leading to errors and reduced performance. We previously developed a machine learning model to estimate response speed in the Psychomotor Vigilance Test (PVT)—quantified as reciprocal reaction time (RRT)—using 4 minutes windows of eyelid opening degree measured by non-contact eye-tracking cameras under daily sleep conditions. The model was developed using a Gradient Boosting Decision Tree with 5-fold cross-validation across 100 randomized runs. The intraclass correlation coefficient (ICC) between the estimated RRT and the observed RRT was 0.83 (95% CI: 0.825–0.841) (Noguchi et al., Worldsleep2025). We aimed to evaluate estimation performance under total sleep deprivation. Because the PVT does not require eye movements, we also tested whether estimated PVT RRT similarly decreases during a visual search task (VST) involving eye movements under sleep deprivation. In a validation experiment, 18 participants completed a 10-minute PVT and an 8-minute VST before and after an 8-hour normal sleep night and a night of total sleep deprivation. Applying the daily-sleep–trained model yielded ICC = 0.52 between observed and estimated PVT RRT, indicating moderate agreement. In the PVT, both observed and estimated PVT RRT significantly decreased after sleep deprivation ($p < 0.001$). In the VST, observed VST RRT and estimated PVT RRT also significantly decreased ($p < 0.001$). Thus, despite its moderate accuracy, the model demonstrated applicability under sleep deprivation and in eye-movement tasks. Incorporating sleep deprivation data into training set may further improve model performance in future studies.

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Exploration of Physiological Markers Sensitive to Sleep Restriction during the Maintenance of Wakefulness Test

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Introduction:
Sleepiness due to sleep deprivation or sleep disorders impairs performance and increases accident risk. Objective tools such as the Maintenance of Wakefulness Test (MWT) are used to detect excessive

daytime sleepiness, but they require specialized facilities, long testing durations, and high costs. More accessible and efficient assessment methods are needed. In this research, we aim to identify electroencephalogram (EEG) and ocular markers sensitive to sleep restriction during the MWT.

Materials and Methods:

Healthy adults (N = 19; 11 females; mean age = 25.4 ± 4.5 years) completed two sleep conditions (8 h normal vs. 4 h restricted) after an adaptation night. Four MWT sessions were performed at 2-hour intervals. High-density EEG and ocular data were collected. EEG analyses included microsleep frequency, microsleep latency, and Hori's EEG stages. Ocular measures included PERCLOS and estimated Psychomotor Vigilance Test (PVT) response speed derived from eyelid dynamics.

Results:

Sleep restriction significantly reduced MWT sleep latency (p = 0.002, d = 0.85) and increased microsleep frequency (p = 0.003, d = 1.03). Percentages of Hori stages 2, 3, and 4 were higher after restriction (p = 0.007, 0.0003, and 0.04; d = 0.99, 1.21, and 0.74). Estimated PVT response speed declined (p = 0.02, d = 0.85), whereas PERCLOS showed no significant change (p = 0.07, d = 0.62).

Conclusions:

Hori stage percentages were the most sensitive markers of sleep restriction. Devices capable of detecting Hori stages may enable practical, real-world sleepiness monitoring.

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X-PAI Funded Project: AI-Powered Sleep Disorder Assessment System Using Awake-state Behavioral Analysis

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Sleep disorders affect over 1 billion people globally, significantly impacting health, productivity, and quality of life. Current sleep medicine is constrained by labor-intensive polysomnography (PSG) diagnostics, limited access to specialized facilities, and high rates of undiagnosed cases (90% remain undiagnosed).

We have been awarded funding from the Cross-Pacific AI Initiative (X-PAI) (Amazon-NVIDIA partnership) to develop an innovative AI-powered sleep disorder assessment system. This collaborative project between University of Tsukuba and University of Washington aims to revolutionize sleep diagnostics through explainable AI that analyzes behavioral patterns during wakefulness.

Our proposed system will identify Sleep Apnea by analyzing facial expressions, speech patterns, and response latency during awake states. The project leverages complementary strengths: Tsukuba's comprehensive clinical data from Mates Clinic and expertise in sleep medicine, combined with Washington's capabilities in real-time AI systems, facial image analysis, and multimodal signal processing.

The system will employ an AI model to integrate multimodal behavioral signals. Key features include: (1) explainable AI with visualization tools allowing clinicians to understand decision pathways, (2) validation against gold-standard PSG data targeting >80% diagnostic concordance.

This project addresses X-PAI focus areas of Health, Aging, and Longevity while incorporating Trustworthy and Explainable AI principles. Planned deliverables include open-source software tools, validated diagnostic algorithms, and a scalable framework for accessible sleep medicine worldwide. This poster presents our project design and implementation strategy for this Japan-US collaborative research initiative.

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Metabolic health and disease progression in Japanese narcolepsy patients: A longitudinal matched cohort study

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Narcolepsy is a central hypersomnia disorder characterised by excessive daily sleepiness, and is

associated with metabolic and cardiovascular comorbidities, such as diabetes and dyslipidemia. While studies primarily reported cross-sectional evidence of such associations, there is a lack of longitudinal data analysis for metabolic changes and complications developed after narcolepsy diagnosis. Using the JMDC claims database records between 2005 and 2024, we examined a longitudinal follow-up cohort of patients with narcolepsy (n = 9,559), defined as two or more claims records corresponding to ICD-10 code G47.4. From the JMDC database, we sampled non-narcoleptic controls matched to narcolepsy patients with a 10:1 ratio by sex, cohort entry and birth month. Metabolic disorders (diabetes and dyslipidemia) are considered present when both relevant diagnoses and medication prescriptions were recorded. There is a significantly higher risk of incident metabolic comorbidities after narcolepsy diagnosis in exposed patients than the age- and sex-matched non-narcolepsy controls, adjusting for age at cohort entry (hazard ratios: diabetes = 2.34 [95%CI 2.08-2.63], dyslipidemia = 2.25 [95%CI 2.08-2.43], $p < .001$). Additionally, mixed-effect models of annual health check-up data were consistent, showing worse and deteriorating metabolic health in narcolepsy patients, such as higher body mass index (+0.62 kgm^{-2}) and low-density lipoprotein levels (+1.52 mg/dL) at age 30. As narcolepsy onset could be at a relatively young age, a better understanding of how and when complications develop after narcolepsy diagnosis informs better strategies to manage such comorbidities, to provide for a higher quality of life and long-term survival in narcolepsy patients after diagnosis.

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Assessment of the Impact of Sleep Scoring on Arousal Detection Using Multitask and Transfer Learning in U-Net Models for In-Home EEG Signals

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Arousal scoring involves detecting subtle, transient EEG frequency changes during sleep and is a key indicator of sleep fragmentation. While automatic arousal detection has been widely explored in polysomnography (PSG), its use in in-home EEG remains limited due to fewer available signals, making detection more difficult. Incorporating sleep stage information—given its association with arousal patterns—offers a promising solution. This study investigates multitask and transfer learning frameworks that integrate sleep scoring to improve arousal detection. We evaluate three U-Net–based architectures—SAAS U-Net, U-Time, and DeepSleep—on two in-home EEG datasets involving healthy individuals and patients with obstructive sleep apnea (OSA). Results show that multitask and transfer learning significantly improve arousal detection in SAAS U-Net and U-Time compared to single-task models. While DeepSleep showed limited gains under an epoch-wise scoring setup in healthy data, experiments with OSA data or its native point-wise resolution revealed multitask learning improved performance over single-task learning. These findings highlight the value of integrating sleep stage classification as an auxiliary task to enhance arousal detection, particularly in-home EEG settings.

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Robust and Interpretable Sleep Stage Scoring for Inter-Individual Variability

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Objective:
This study aimed to demonstrate that introducing a Transformer architecture enhances robustness to inter-individual variability compared to conventional models, and to explicitly interpret and analyze the underlying mechanisms of this robustness from a clinical perspective.
Methods:
Two publicly available EEG datasets were used to evaluate the performance of the Transformer-based model. First, we analyzed the subject-wise distributions within the feature space extracted by a CNN. Then, by examining the attention weights of the Transformer in detail, we clarified how the model captures individual characteristics.

Results:
The proposed model formed distinct subject-level clusters, particularly in the N2 and N3 stages, reflecting the physiological diversity emphasized by clinicians. Furthermore, analysis of attention weights revealed that the model adaptively emphasized both universally important and subject-specific features. This dual strategy closely resembled the way clinicians combine "general judgment" with "individualized assessment" in practice.
Conclusion:
This study demonstrated that achieving both robustness to inter-individual variability and medical interpretability is essential in automatic sleep stage classification models. The findings represent an important step toward developing an automated scoring system that can be trusted in clinical practice.

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Elucidating REM sleep regulation through the *Dreamless* mutation in mice

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Although REM sleep (REMS) is ubiquitous in mammals, the molecular and neural mechanisms underlying REMS regulation remain unknown. We previously established the *Dreamless* mutant pedigree, which exhibits abnormal EEG power spectra and an approximately 50% reduction in REMS total time and fragmented REMS episode duration. We identified an SNP specific to *Dreamless* mutant mice within the *Nalcn* gene, which leads to a single amino acid substitution (N315K) in NALCN, a monovalent cation leak channel. This finding suggested an important role of NALCN in REMS regulation.
To elucidate the brain regions and neuronal subtypes through which NALCN regulates REMS, we generated $Nalcn^{flox}$ conditional knock-out (cKO) and $Nalcn^{Drl\text{-}FLEx}$ conditional knock-in (cKI) mice bearing Cre-dependent loss-of-function and gain-of-function *Nalcn* alleles, respectively. In *Nalcn* cKI mice, crosses with systemic Cre-expressing lines phenocopied the *Dreamless* mutant. Crossing with *Foxg1-IRES-Cre* (expressed in telencephalic primordium) or *Emx1-Cre* (expressed in developing cortical excitatory neurons) lines, both cKI and cKO mutants showed theta/delta dissonance and reduced episode duration during REMS. On the other hand, in *En1-Cre* (developing midbrain/hindbrain junction) cKO, fragmentation of REMS was observed. These results suggest that NALCN in both the telencephalon and midbrain-pons region is required for the maintenance of REMS, and that NALCN in cortical excitatory neurons plays a key role in the regulation of REMS.

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REM sleep regulation through KCTD family

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Sleep is an evolutionarily conserved behavior observed across species, yet its molecular regulatory mechanisms remain incompletely understood. To investigate the mechanisms underlying sleep–wake regulation, we performed a forward genetic screening and identified that deletion of exon 13 in SIK3 leads to increased NREM sleep duration and elevated sleep need. To further explore the molecular mechanism of SIK3-mediated sleep regulation, we analyzed the SIK3 interactome using immunoprecipitation followed by LC–MS/MS. As a result, we identified several KCTD family proteins that interact with SIK3. The KCTD family members are known adaptor proteins of E3 ubiquitin ligases that mediate substrate recognition. Although their roles in mammalian sleep–wake regulation have not been elucidated, homologs such as *insomniac* in *Drosophila* are known to cause a marked reduction in sleep when disrupted.
We therefore examined sleep and wakefulness in mice deficient for two SIK3-interacting KCTD proteins. Conditional deletion of these genes in neurons in late infancy resulted in a significant increase in NREM sleep time with elevated EEG delta power, while REM sleep was markedly reduced. Similarly, deletion of these genes in *Vglut2*-positive excitatory neurons caused a marked increase in NREM sleep duration with enhanced delta power and a significant reduction in REM sleep.

In contrast, deletion in *ChAT*-positive neurons increased NREM sleep time without affecting REM sleep.
These findings suggest that the two KCTD proteins play essential roles in regulating REM sleep and may function downstream of SIK3 in sleep–wake control.

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The SIK3-GEF-H1-RhoA signaling pathway regulates sleep need

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Sleep behavior is conserved across species and is regulated by the accumulation and dissipation of sleep need. Despite its importance, the molecular and cellular basis of sleep need remains unclear. Our recent studies suggest that signaling pathways centered on SIK3 play an important role in regulating homeostatic sleep need (Funato et al., Nature, 2016; Kim et al., Nature, 2022). To elucidate the molecular basis of homeostatic sleep need, we identified the SIK3 downstream signaling pathway.
Through in vitro substrate screening, we identified Rho guanine nucleotide exchange factor, GEF-H1 as a novel SIK3 substrate. Our biochemical analysis revealed that SIK3 activates GEF-H1 by releasing it from microtubules via phosphorylation. Furthermore, expressing a constitutively active form of GEF-H1 (caGEF-H1) throughout the brain increased NREM delta power, an indicator of sleep need. This phenotype is consistent with the phenotype of the constitutively active SIK3 mutant (Sleepy). GEF-H1 activates RhoA, one of the Rho GTPases. Thus, we knocked down RhoA in mice expressing caGEF-H1 and found that RhoA knockdown suppressed increased NREM delta power induced by caGEF-H1. These results suggest that the SIK3-GEF-H1-RhoA pathway enhances sleep need.
Rho GTPases are known regulators of synaptic plasticity. Therefore, we speculated that SIK3 regulates sleep need by controlling synaptic strength via RhoA. As expected, Sleepy mice showed increased spine volume compared to wild-type mice, suggesting that SIK3 positively regulates synaptic strength. Taken together, these results suggest that the SIK3-GEF-H1-RhoA pathway regulates sleep need by controlling synaptic strength.

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The *Dreamy* gene, identified by forward genetics screening, bi-directionally regulates quantity of rapid eye movement sleep in mice

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Rapid eye movement (REM) sleep is characterized by vivid dreaming, wake-like electroencephalogram (EEG) patterns, skeletal muscle atonia, and activation of the autonomic nervous system. Over the past decade, key neuronal populations regulating REM sleep have been identified. However, molecular mechanisms underlying the regulation of REM sleep remain elusive. To address this, we have conducted large-scale, phenotype-driven, forward genetic screening in mice and have recently established a novel pedigree, *Dreamy* (*Dmy*), displaying an increased REM sleep amount.
We generated genome-edited mice carrying the *Dmy*-mimic point mutation in the *Dreamy* gene, identified through linkage analysis and whole-exome sequencing. Heterozygous *Dreamy*$^{Dmy/+}$ mice showed an increased REM sleep amount with higher EEG theta power during REM sleep. To further investigate whether the increased REM sleep in *Dmy* is due to a gain-of-function or loss-of-function phenotype, we employed AAV-B10-based genetic manipulations of the *Dreamy* gene in neurons throughout the brain. AAV-mediated overexpression of C-terminally truncated *Dreamy* gene significantly decreased a total REM sleep time. By contrast, neuron-specific knockdown of the *Dreamy* gene using AAV-based multi-shRNAs increased a total REM sleep time with higher EEG theta power during REM sleep. These data suggested that the increased REM sleep in *Dmy* results from the loss-of-function phenotype of *Dreamy*.
In summary, our study identifies the *Dreamy* gene as a crucial factor in the regulation of REM sleep and provides novel insights into the molecular mechanisms that govern REM sleep.
