Poster Session Abstracts

The 5th Annual IIIS Symposium・The 32nd Wako Workshop・Joint Meeting

Data Blitz by Poster Presenters	**14:20 – 14:45**
Poster Session	**14:45 – 16:00**

Poster list

P01

Orexin acts on the locus coeruleus to enhance fear expression

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Salient emotional information activates orexin neurons in the lateral hypothalamus (LH-OX neurons), leading to increases in arousal and autonomic function. However, how this circuit alters animals' behavior remains unknown. We found that noradrenergic neurons in the locus coeruleus (LC-NA neurons) which project to the lateral amygdala (LA) receive direct presynaptic input from LH-OX neurons. Pharmacogenetic or optogenetic silencing of this circuit inhibited sustained expression of fear responses, as did acute blockade of the orexin receptor-1 (OX1R) by an antagonist administered just before the test session. In contrast, optogenetic stimulation of the LH→LC→LA circuit after fear conditioning induced freezing behavior in a similar but distinct context. Furthermore, upregulating orexinergic tone by fasting also enhanced freezing behavior. These findings demonstrate that the LH→LC→LA pathway plays an important role in the regulation of fear-related behavior in response to environmental stimuli, and dysfunction of this circuit may underlie inappropriate generalization of fear. Our study also suggests that the inhibition of OX1R is a promising avenue for treating psychiatric conditions that are characterized by exaggerated and/or inappropriate fear-related responses triggered by external cues, such as panic disorder and PTSD.

P02

Neuronal firing in the cerebral cortex during waking and NREM sleep

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Cortical networks exhibit profoundly different activity patterns in sleep compared to waking. While it is safe to assume that cortical activity during waking is underlying information processing, the function of cortical activity patterns in NREM sleep is largely unknown. It might underlay memory consolidation, synaptic rebalancing or a number of other functions.

In NREM sleep cortical neurons show synchronous transitions between silent down- and active up-states – a profound shift in temporal patterning. We were interested in determining how this global temporal pattern interacted with local patterns of neuronal activity. We measured single unit (SU), multi-unit (MU) and local field potential (LFP) activity in mice using multiple tetrodes inserted into the cortex. We found that though total number of spikes in cortical neurons did not differ between waking and NREM sleep, spiking pattern was closely locked to LFP slow wave, showing cluster of spikes in active phase and silence of spikes in silent phase. Entropy analysis of ensemble of neurons showed higher entropy in NREM sleep than in waking, indicating that neurons were less organized during NREM sleep. To further elucidate a role of NREM sleep, intracellular calcium concentration by spiking was calculated. Peak calculated calcium influx was higher in NREM sleep than in waking, which was similar to results from two-photon calcium imaging from waking and sleeping animal. These results indicate that cortical networks observed during waking become weaker and intracellular calcium increases during NREM sleep.

P03

Stimulation of α2-adrenergic receptors limits the options explored by prospective spatial representations in hippocampal neural activity

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How much one deliberates about the choice before taking an action is an important aspect of decision-making. A series of studies have suggested that noradrenaline plays a role in regulation of deliberation. For example, our previous study reported that clonidine, an α2-adrenergic auto-receptor agonist, makes animals less deliberative/more decisive, reflected as a decrease in deliberation-related head re-orienting behavior of rats (vicarious trial-and-error [VTE]). VTE is known to be a hippocampus dependent behavior. However, the mechanisms by which clonidine makes animal less deliberative/more decisive remain unknown. Here, we investigate the neural mechanisms in hippocampus underlying deliberation by examining effects of systemic injection of clonidine on hippocampus CA1 neural activity in T-maze choice task.

Consistent with earlier experiments, clonidine decreased VTE. Hippocampal representations were assessed by applying a one-step Bayesian spatial decoding algorithm to the recorded neural ensemble activity. Under vehicle control, at the choice point, hippocampal spatial representation reflected both the chosen and unchosen paths in VTE laps, implying that deliberation explored both choices, while hippocampal representation reflected only the chosen path in non-VTE laps. Clonidine suppressed hippocampal representation of the unchosen path even in VTE laps as well as in non-VTE laps, suggesting that clonidine limits mental search. Our findings suggest that deliberation underlies hippocampal search processes of alternative options in a process which can be modulated by noradrenaline. These data also suggest that noradrenaline plays a role in balancing exploration and exploitation in internally simulated behaviors, similar to its role in balancing exploration and exploitation in external behaviors.

P04

Chronic orexin receptor blockage induces cataplectic behavior by reducing orexin peptide synthesis

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Orexins/hypocretins are key neuropeptides responsible for regulating central arousal and reward circuits. Two receptors respond to orexin signaling, orexin 1 receptor (OX_1 R) and orexin 2 receptor (OX_2 R) with partially overlapping nervous system distributions. Various orexin receptor antagonists have been developed as a therapy for insomnia and other disorders with disruption of sleep and wake. One such example is suvorexant (MK-4305), a potent, selective, and orally bioavailable antagonist of OX_1 R and OX_2 R, recently approved for human use in Japan and USA. On the other hand, it is well known that disruption of orexin signaling leads to narcolepsy in humans, and orexin knockout mice also develop cataplexy. Hence, we hypothesize that chronic antagonism of orexin receptor with high dose can lead to down-regulation of orexin system mimicking partial or complete orexin-KO condition. We found that chronic suvorexant administration in mice followed by 1-week washout and re-challenge with similar dose leads to cataplexy. As expected, it increases amount of NREM as well as REM sleep, acutely. The sleep-inducing effect of suvorexant was reduced on its chronic use. Further, orexin peptide synthesis decreased and OX_2 R increased by chronic suvorexant administration in mice. Putting this data together, we concluded that chronic orexin receptors blockage leads to reduced orexin peptide synthesis that results in cataplectic behavior in mice.

P05

Development of cell therapy model for narcolepsy

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Narcolepsy is a sleep disorder, which is characterized by the instability of sleep/wake control. This disease is caused by a massive loss of orexin neurons producing a neuropeptide, orexin, in the lateral hypothalamus. Current treatment options for narcolepsy patients are limited to symptomatic drug therapy and lifestyle improvement. Therefore, a new approach targeting orexin system is required as an etiological treatment for narcolepsy.

Previous studies have been shown that the replacement of orexin ameliorates the pathological condition. For example, the intracerebroventricular (i.c.v.) administration, ectopic overexpression of orexin and systemic administration of orexin receptor agonist suppress cataplexy-like behaviors and improve other sleep abnormalities in narcoleptic mice. Yet, the effects of these approaches are transient or need genetic modification. In contrast, there is a concept of cell therapy for narcolepsy; orexin neurons are transplanted into the brain and supply orexin as long as life lasts.
However, there is no report certainly proving whether the concept of cell therapy for narcolepsy works or not. We aimed at giving an evidence on the therapeutic effectiveness of neural transplantation for narcolepsy.
As a first step, we developed an experimental system for cell therapy model. In this system, orexin neurons harvested from various sources are transplanted to narcoleptic mice followed by the recordings of electroencephalography and electromyography to judge the effect on sleep phenotype. By using this system, we started the screening for the necessary condition of the therapeutic effectiveness. In this presentation, we will show the overview of the experimental scheme and the preliminary data.

P06

Dual and orexin 2 receptor antagonists induce REM sleep via mechanisms beyond noradrenergic LC signaling

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Antagonism of orexin receptors (OX1R, OX2R) induce sleep architecture characterized by increases in NREM and REM reminiscent of unmedicated sleep, and provide an alternative to GABA-A receptor modulators. REM sleep is thought to be controlled in part by noradrenergic neurons of the locus coeruleus (LC), a site of selective OX1R expression. Dual orexin receptor antagonists (DORAs) and OX2R-single antagonists (2-SORAs) effectively promote somnolence, but their differential effects on sleep architecture, particularly REM sleep remains unclear. This work utilizes selective DORA and 2-SORA treatments in genetic models and in combination with prazosin (α1-adrenergic blocker), to determine the specific roles of OX1R versus OX2R important for sleep architecture changes induced by DORAs and 2-SORAs. In result, NREM and REM sleep induced by DORAs is statistically no different from un-medicated inactive phase sleep in rats, unlike GABA modulators, which reduce REM sleep in rodents and even promote paradoxical arousal in dogs. Genetic models demonstrate that orexin-mediated arousal is predominately mediated by OX2R, while OX1R appears involved in vigilance state gating. REM sleep induced by DORAs and 2-SORAs, however, was similarly augmented by prazosin, indicating that noradrenergic signaling from the LC is not maximally inhibited by either treatment. In rats, DORAs required lower receptor occupancy relative to 2-SORAs to induce sleep. MK-1064, a selective 2-SORA (3000x OX2R/OX1R selective binding) induces REM sleep across mammalian species.

P07

The role of Ca^{2+}-dependent hyperpolarization pathway underlying sleep-duration regulation

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The detailed molecular mechanisms underlying sleep-duration regulation in mammals are still elusive. To address this challenge, we constructed a simple computational model of an averaged neuron, which recapitulates the electrophysiological characteristics of the slow-wave sleep and awake states. Comprehensive bifurcation analysis of an ensemble of more than 1,000 models predicted that a Ca^{2+}-dependent hyperpolarization pathway may play a role in slow-wave sleep and hence in sleep-duration regulation. To experimentally validate the prediction, we combined the triple-target CRISPR method and the non-invasive sleep/wake recording system SSS to comprehensively generate and analyze 21 KO mice. We found that impaired Ca^{2+}-dependent K^{+} channels (*Kcnn2* and *Kcnn3*) or voltage-gated Ca^{2+} channels (*Cacna1g* and *Cacna1h*) decrease sleep duration. Pharmacological intervention and whole-brain imaging of neural activity with single-cell resolution validated that impaired NMDA receptors reduce sleep duration and directly increase the excitability of cells. We further validated that impaired plasma membrane Ca^{2+} ATPase (*Atp2b3*) increases sleep duration while impaired Ca^{2+}/calmodulin-dependent kinases (*Camk2a* and *Camk2b*) decreases sleep duration. Based on these results, we propose a hypothesis that a Ca^{2+}-dependent hyperpolarization pathway underlies sleep-duration regulation in mammals.

P08

Serotonergic neurons of the dorsal raphe mediate anti-cataplectic action of orexin neurons by reducing amygdala activity

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Narcolepsy is a sleep disorder caused by the loss of orexin (hypocretin)-producing neurons. The cardinal symptoms include excessive daytime sleepiness and a sudden weakening of muscle tone (cataplexy), which is often triggered by strong emotions. We previously demonstrated that serotonin neurons of the dorsal raphe nucleus (DRN) mediate the suppression of cataplexy by orexin neurons in a mouse model of narcolepsy. Using an optogenetic tool, we show here that acute activation of DRN serotonin neuron terminals in the amygdala, but not in nuclei involved in the regulation of REM sleep and atonia, suppressed cataplexy. The amygdala activity was reduced by the stimulation of serotonin nerves. Furthermore, pharmacogenetic inhibition of the amygdala using designer receptors exclusively activated by designer drugs (DREADDs) drastically decreased cataplexy, while its activation increased cataplexy. Lastly, optogenetic inhibition of nerve terminals in the amygdala cancelled the anti-cataplectic effects of orexin signaling in DRN serotonin neurons. Collectively, these results suggest that DRN serotonin neurons, as a downstream target of orexin neurons, inhibit cataplexy by reducing the activity of amygdala, which is a center for emotional processing.

P09

New developments in AI-based automatic sleep staging and apnea events detection using information geometry and deep learning methods

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The author summarizes latest results of automatic sleep apnea detection and staging, which has been obtained using information geometry-derived, as well as newly developed artificial intelligence (AI) machine learning methods. The discussed AI applications are realized using shallow and deep learning-based classification methods. The presented automatic sleep apnea detection and staging technique is a further extended method, which is a fusion of data-driven signal processing utilizing a synchro-squeezing-transform (SST) preprocessing and AI. In the presented project the multimodal physiological signals such as: EEG, EMG, EOG, ECG, oro-nasal airflow, ribcage and abdomen movements, oxygen saturation (pulse-oximetery), acoustic snoring, and body position are classified with improved accuracy comparing to previous reports by the author. For home-use oriented as well as clinical sleep monitoring applications a plug & play operation is usually considered as a requirement for any AI-based devices. The presented results are a step forward towards a development of wearable sleep monitors. The current project results have been also improved for the best accuracies using the combined brainwave and body peripheral signals for the both, automatic sleep apnea detection and staging. The results with AI-based approach thus further support our hypothesis of the data-driven bio-inspired signal processing and information geometry-based feature extraction approach validity for a very hot topic of a sleep wellness improvement.

P10

Thalidomide action on CNS activity

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Thalidomide was developed in the 1950s as an extremely safe sedative. With no addictive potential or risk of accidental overdose, it was proclaimed a “wonder drug” for providing safe and sound sleep. Unfortunately, it is highly teratogenic in man. While the mechanism for its teratogenic, anti-proliferative and immunomodulatory actions have been well revealed, its hypnotic effects have not been systematically studied. Here we report that thalidomide maintains its sedative properties even if the teratogenic pathway is blocked by the mutation of its target, cereblon. This provided us with the motivation to uncover the pharmacodynamics of thalidomide-mediated sedation. Thalidomide had no effect on inhibitory synaptic transmission in cortical neurons, but depressed spontaneous and evoked excitatory synaptic transmission in cortical slice preparations.

P11

Zinc-rich oysters as well as zinc yeast- and astaxanthin-enriched food improved sleep efficiency and sleep onset in a randomized controlled trial of healthy individuals

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Scope: Zinc is an essential mineral that plays an important role in the body. We previously reported that orally feeding zinc-enriched yeast to mice induces non-rapid-eye-movement sleep. In addition, astaxanthin, an antioxidant abundant in seafood such as salmon and krill, is able to chelate minerals and may promote zinc absorption, which in return may also improve sleep. The purpose of our study was to examine the effect of zinc-rich and astaxanthin-containing food on sleep in humans.
Methods and results: We conducted a randomized, double-blinded, placebo-controlled parallel group trial of 120 healthy subjects and recorded their night activity by actigraphy for 12 weeks. These subjects were divided into 4 groups: placebo, zinc-rich food, zinc- and astaxanthin-rich food, and placebo supplemented with zinc-enriched yeast and astaxanthin oil. Compared with the placebo group, the zinc-rich food group efficiently decreased the time necessary to fall asleep and improved sleep efficiency, whereas the group that ingested zinc-enriched yeast and astaxanthin oil significantly improved the sleep onset latency.
Conclusion: Actigraphic sleep monitoring demonstrated that eating zinc-rich food improved sleep onset latency as well as improved the sleep efficiency in healthy individuals.

P12

The effects of dark/light transition and sleep-wake cycles on jaw-closing masseter muscle activity level in mice

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Bruxism is characterized by involuntary and exaggerated jaw motor activity during both wakefulness and sleep. However, it is unclear how jaw-closing masseter muscle activity changes over a 24-h period in mice. In this study, we examined EMG activity of the masseter muscle compared with that of the neck muscle during wakefulness (W), non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep using recordings of electroencephalography (EEG), electro-oculography (EOG), and electromyography (EMG) of neck and masseter muscles.

The mean EMG activities of both neck and masseter muscles during W were significantly larger than those during NREM sleep and REM sleep. In contrast, the EMG activities of both muscles slightly, but significantly decreased during the transition period from dark to light. During NREM sleep, EMG activity of the masseter muscle was correlated with that of neck muscle, whereas there was no correlation between both muscles during W and REM sleep. Histogram analysis revealed that the masseter EMG activity during W and NREM sleep showed bimodal distributions, whereas the neck EMG activity showed unimodal distributions in all states. These results suggest that the activities of masseter and neck muscles are modulated by sleep-wake state rather than dark/light transition, and even during NREM sleep, masseter EMG activity also showed bimodal fluctuations, which may involve in the unexpected occurrence of increased masseter EMG activity such as sleep bruxism.

P13

A novel adenosine A_{2A} receptor allosteric modulator promotes sleep in mice

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Sleep is an intensive anabolic state during which the body carries out growth and repair processes such as the formation of new bones, muscles, and other tissues. Adenosine has long been known to represent a state of relative energy deficiency: adenosine triphosphate depletion and the elevation of extracellular adenosine levels are positively correlated and positively associated with sleep. The intracerebroventricular (ICV) infusion of A_{2A} receptor ($A_{2A}R$) agonist CGS21680 induces sleep. Due to the lack of brain-permeability, however, all currently existing $A_{2A}R$ agonists are not suitable for treating the nervous system. Allosteric compounds have higher receptor selectivity than orthosteric ligands, as their action is limited to when and where the endogenous ligands are released. We have established $A_{2A}R$-expressing Chinese hamster ovary cells to measure cAMP produced upon Gs-coupled $A_{2A}R$ activation by using a fluorescence resonance energy transfer (FRET) immunoassay. By screening of several thousand small-molecule compounds in the cell-culture bioassay, we have identified a novel positive allosteric modulator for $A_{2A}R$, termed YNT-378. When we examined the sleep inducing activity of YNT-378 by monitoring electroencephalogram, we found that ICV infusion of YNT-378 (200 nmol/μl) into the brain of mice strongly induced slow wave sleep (SWS) and the intraperitoneal (IP) administration of YNT-378 dose dependently (30–75 mg/kg) increased the total amount of SWS and decreased wakefulness. In addition, $A_{2A}R$ antagonist ZM241385 (15 mg/kg, IP) suppressed the sleep-inducing effect of YNT-378. Pharmacologic $A_{2A}R$ activation may be an alternative strategy for the treatment of insomnia and help people with sleep problems to fall asleep.

P14

Orexin B plays an inhibitory role in noradrenergic neurons in the locus coeruleus

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Sleep and wakefulness are governed by several neuromodulators in the brain, of which the interaction of orexinergic and noradrenergic systems plays a pivotal role in the maintenance of wakefulness. Orexin neurons provide a direct excitatory signal to noradrenergic neurons in the locus coeruleus (LC-NA neurons) through the activation of orexin type-1 receptors (OX1R) by the neuropeptide orexin A. However, the orexinergic regulation of LC-NA neurons seems more complex: for example, ablation of orexin neurons actually increases firing rate of LC-NA neurons in vivo. Here we focused on the possible role of orexin B, an orexin type-2 receptor (OX2R)-selective agonist co-released from orexin neurons. Surprisingly, in contrast to orexin A, orexin B tonically reduced spontaneous firing of LC-NA neurons, which was mediated through $GABA_A$ receptors ($GABA_AR$) and OX2R. OX2R is expressed in GABAergic neurons within and around the LC (LC-GABA neurons). Unexpectedly, orexin B did not appreciably increase excitability of LC-GABA neurons and GABAergic postsynaptic currents in LC-NA neurons, while the peptide induced $GABA_AR$-mediated tonic current and reduced input resistance in LC-NA neurons. These findings suggest that orexin neurons could provide a continuous inhibitory tone to LC-NA neurons via a novel modulatory action of orexin B.

P15

Functional analysis of a novel sleep-related gene, *Sik3* in *Drosophila melanogaster*

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Despite the number of studies reporting factors and genes regulating sleep, the basic mechanism how sleep is controlled, especially which determines the amount of sleep, is still beyond our understanding. In order to approach this problem without a priori assumption, a forward-genetics approach is now underway. Thus far, more than 8000 mice with induced mutations were screened and several sleep-related phenotypes were successfully discovered. One of them, *Sleepy* mutation dramatically increased total sleep time, and *Sleepy* was identified as a skip mutation of exon 13 of the sodium-inducible kinase *Sik3* gene. A homologue of *Sik3* was found in *Drosophila melanogaster*, and in this study, we analyzed its function. Sleep and activity were examined conventionally by recording infrared beam crossing using *Drosophila* Activity Monitor. A hypomorphic *Sik3* mutant fly showed reduced sleep and increased activity. Murine *Sik3* has a serine residue in exon 13, which is phosphorylated by PKA and conserved in *Drosophila Sik3*. It is presumed that the phosphorylation of this serine inactivate SIK3 kinase. Therefore, we examined phosphorylation-defective SIK3 (SIK3-S563A), and found pan-neuronal constitutive over-expression of SIK3-S563A increased sleep. Conditional over-expressions of SIK3-S563A using TARGET system and GeneSwitch system also increased sleep. In order to localize where SIK3 functions in sleep regulation, we expressed SIK3-S563A only in the subset of neurons, and found glutamatergic neurons and octopaminergic neurons were possible candidates. We are also working other functions of *Sik3* and upstream and downstream signals regulating it.

P16

Forward genetics approach in identification of novel sleep/wakefulness related gene(s)

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In a recent report, our group showed successful application of the forward genetic approach in identification of two distinct genes regulating sleep-wakefulness behavior (Funato *et al., Nature* 2016). Through a high-throughput screening of randomly mutagenized mice, several pedigrees showing heritable sleep abnormalities have been established. Here we present new mutant mouse pedigree exhibiting abnormal sleep behavior, named *Sleepy2,* characterized by long NREM sleep time. Whole-exome deep sequencing, combined with a linkage analysis identified a single nucleotide change of A to T in the splicing acceptor site. The *Sleepy2* gene family shares highly conserved domain at which the splicing mutation is located, proposing a conserved role of the gene family in regulation of sleep-wakefulness behavior.

Using the CRISPR/Cas9 technology, the splicing mutation was reproduced in all 4 members of the *Sleepy2* gene family to prove causal relation between the splicing mutation and perturbed mutation. First in *Sleepy2*O pedigree (initially identified through the ENU-mutagenized study), the polysomnography analysis based on EEG/EMG recording showed longer NREMS as observed in the ENU-mutagenized founder mice. Interestingly, two other members of the gene family, *Sleepy2F* and *Sleepy2N* also showed longer NREM sleep time. Further investigation and scrutiny of *Sleepy2* mutant pedigree may reveal novel modulators of the system and help us gain fundamental understanding of sleep.

P17

Optogenetic silencing of adult-born neurons during sleep impairs memory consolidation

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Mammalian sleep contains rapid eye movement (REM) and non-REM sleep, both could employ different mechanisms for memory consolidation. Previous reports showed that memory-associated odor stimulation during non-REM sleep enhanced memory consolidation[1]. In addition, boosting slow oscillations or inhibiting sharp wave-ripples during non-REM sleep potentiated or interfered with memory consolidation[2,3]. On the other hand, REM sleep deprivation inhibited memory consolidation[4]. However, the memory circuit that is responsible for memory consolidation during each sleep stage has not been clearly shown. We have shown that hippocampal adult-born neurons are incorporated in memory circuits after learning[5]. Therefore, we silence the activities of the adult-born neurons during specific stages of sleep after learning using optogenetics, which provides reversibility in intervention with higher time resolution and target specificity. The intervention reveals that the activities of the adult-born neurons is necessary for memory consolidation during specific stage of sleep.

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P18

Molecular basis of innate fear: from fear genes to fear/anxiety disorders

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Fear is a basic emotion that enhances animal survival by triggering the "fight, flight, or freeze" response to perceived danger. For example, lab mice will freeze instantly at the first sight of a cat, despite being raised away from predators for hundreds of generations. Thus, this fear response is "innate", rather than "learned", and thus must be genetically encoded. Uncontrolled fear is closely linked to various mental disorders, such as phobia, post-traumatic stress disorder (PTSD), depression, obsessive-compulsive disorder (OCD) etc. However, very little is known about the molecular basis of fear. To this end, we have developed a robust predator odor-based "innate fear" assay and conducted a forward genetic screen in N-ethyl-N-nitrosourea (ENU)-mutagenized mice to search for "fear" genes. To date, we have successfully established two pedigrees with heritable abnormal fear behaviors, and identified one causative mutation after screening ~1600 ENU mice. We believe that this unbiased fear screen will identify core fear genes and elucidate the molecular mechanisms of fear. Further, these studies will break new grounds for us to develop novel therapeutic treatments for fear-related mental disorders.