1-YIA-01 YIA

Chronic histamine decrease in adult mice induced depression-like behavior and impaired sleep-wake cycle

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Histamine acts as a neurotransmitter in the brain. Histamine is synthesized from histidine by histidine decarboxylase (HDC). In the central nervous system, HDC-positive neurons exist in tuberomammillary nucleus (TMN) of posterior hypothalamus and project their axons to entire brain. Recent studies showed that chronic histamine decrease in adult was observed in several neurological disorders such as narcolepsy and Alzheimer's disease. However, it is still unknown whether this histamine decrease plays a causative role in the disorders or not. In present study, we induced chronic histamine deficiency in adult mice to reveal the direct involvement of impaired histaminergic nervous system in brain dysfunction. We stereotaxically microinjected adeno-associated virus expressing
Cre-recombinase into TMN of adult HDC flox mice (HDC cKO mice) for long-term brain histamine decrease. Immunohistochemical analysis showed Cre expression in TMN in HDC cKO mice. We confirmed the reduced HDC mRNA expression and the decreased histamine content in HDC cKO brain. In the tail suspension test, immobility time was prolonged in HDC cKO mice. In home-cage locomotor activity test, activity counts during light period were decreased in HDC cKO mice. Additionally, we performed sleep analysis by measuring electroencephalogram and electromyogram. The analysis showed that cKO mice exhibited decreased wakefulness during light period. These results indicated that chronic dysfunction of histamine system caused depression-like behavior and impaired sleep-wake cycle in mice.

1-YIA-02 YIA

Chronic pain-induced plastic change in the extended amygdala neural circuit causes maladaptive anxiety

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Chronic pain is often comorbid with psychiatric disorders such as depression and anxiety disorders, suggesting common neuronal mechanisms that underlie these pathological states, yet the mechanisms are still unclear. Here, using spared nerve injury model mice of chronic pain, we show the neuroplastic changes in the synaptic transmission in the bed nucleus of the stria terminalis (BNST) neurons projecting to the lateral hypothalamus (LH) which can lead to maladaptive anxiety. Consistent with previous reports, chronic pain increased anxiety-like behaviors in elevated plus maze and light-dark box tests. Whole-cell patch-clamp recordings revealed that chronic pain increased spontaneous IPSCs onto I_h-current negative LH-projecting BNST neurons. To test the causal role of this plasticity in behavioral anxiety, we inhibited or activated the LH-projecting BNST neurons using a DREADD technique and accessed anxiety-like behaviors. Inhibition of LH-projecting BNST neurons in naïve mice increased anxiety-like behaviors without affecting nociceptive behaviors. Furthermore, activation of LH-projecting BNST neurons ameliorated anxiety-like behaviors in chronic pain model mice. Collectively, these findings suggest the critical role of sustained suppression of the LH-projecting BNST neurons in maladaptive anxiety during chronic pain.

1-YIA-03 YIA

Analysis of ultrastructural alterations in the mouse medial prefrontal cortex toward the understanding of pathophysiology of social stress-induced depressive-like behaviors

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Animal studies using various stress models have shown that excessive environmental stress induces depressive-like behaviors with concomitant atrophic changes of neurons especially in the medial prefrontal cortex (mPFC) and in the hippocampus. Despite the accumulating evidences showing that multiple cellular and molecular events including persistent increase of the corticosteroid hormone and brain inflammation are inducers of this neuronal atrophy, its underlying molecular mechanisms particularly inside the neurons are still elusive. In the present study, we aimed to examine the ultrastructural events inside neurons in the mPFC during the course of chronic social stress in mice. We subjected male C57BL/6 mice to either single or repeated social defeat stress and analyzed the brains from those stressed mice or from control mice which did not receive defeat stress by serial electron microscopy. We found that social stress induced disruptions of plasma membranes of neuronal dendrites in the mPFC with concomitant morphological alterations of subcellular organelle and cytoskeletons. We will discuss the potential biological insights and mechanisms of these ultrastructural events.

1-YIA-04 YIA

KNT-127, a selective delta-opioid receptor agonist, exhibits antidepressant effects through PI3K-Akt-mTOR signal transduction in the mice prefrontal cortex.

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Recently, it has been suggested that mammalian target of rapamycin (mTOR) signaling in the prefrontal cortex (PFC) plays key roles in the molecular mechanisms of antidepressant-like effects in rodents. We previously reported that a selective delta-opioid receptor (DOP) agonist, KNT-127 produced robust antidepressant-like effects in the forced swimming test (FST) in mice. However, the detailed mechanism of its effect has remained elusive. Therefore, we attempted to identify the molecular mechanism of the antidepressant-like effects of KNT-127 using the mouse FST. We firstly demonstrated that a selective mTOR inhibitor rapamycin (i.c.v.) significantly diminished the antidepressant-like effects of KNT-127 (s.c.) in the FST. In addition, a selective PI3 kinase inhibitor LY294002 (i.c. v.), which inhibits the upstream molecules of mTOR, also diminished the antidepressant-like effects of KNT-127. Furthermore, a protein immunoblotting assay revealed that KNT-127 (s.c.) increased the level of phosphorylation of Akt in the mouse PFC. Taken together, we proposed that KNT-127 produced the antidepressant-like effects in the FST via the activation of PI3K-Akt-mTOR signal transduction pathway in the PFC in mice. These results take the first step on the way to elucidate the mechanical functions of DOP agonists for antidepressants.

1-YIA-05 YIA

Roles and mechanisms of social defeat stress-induced prostaglandin E_2 synthesis in the mouse brain

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Prolonged or excessive stress caused by social environment induces psychological and physiological alterations, and increases a risk for psychiatric disorders. In repeated social defeat stress (SDS), a rodent model to study psychiatric disorders, we previously reported that prostaglandin (PG) E_2, an inflammation-related lipid mediator, and toll-like receptor (TLR) 2/4, innate immune receptors, are crucial for repeated SDS-induced social avoidance. However, the mechanism of SDS-induced PGE_2 synthesis in the brain and the involvement of TLR2/4 remain unknown. Here we show that SDS increased the PGE_2 contents in subcortical brain regions of wild-type mice, and that this increase was abolished by perturbations of TLR2/4, COX1 and COX2. It has been reported that free arachidonic acid for PGE_2 synthesis in the brain is supplied from monoacylglycerol lipase (MAGL)-mediated metabolism of endocannabinoid 2-arachidonoylglycerol. Consistently, systemic administration of JZL184, a MAGL inhibitor, inhibited SDS-induced PGE_2 synthesis in subcortical regions and social avoidance. These results suggest that SDS induces PGE_2 synthesis derived from 2-arachidonoylglycerol via the MAGL-COX pathway in subcortical regions to induce social avoidance, and that this PGE_2 synthesis is maintained by TLR2/4 activity.

1-YIA-06 YIA

8-Hydroxylation and glucuronidation of mirtazapine in Japanese psychiatric patients: Significance of the glucuronidation pathway of 8-hydroxy-mirtazapine.

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OBJECTIVE To investigate the metabolism of mirtazapine (MIR) in Japanese psychiatric patients, we determined the plasma levels of MIR, *N*-desmethylmirtazapine (DMIR), 8-hydroxy-mirtazapine (8-OH-MIR), mirtazapine glucuronide (MIR-G), and 8-hydroxy-mirtazapine glucuronide (8-OH-MIR-G). **METHODS** Seventy-nine Japanese psychiatric patients were treated with MIR for 1–8 weeks to achieve a steady-state concentration. Plasma levels of MIR, DMIR, and 8-OH-MIR were determined using HPLC. Plasma concentrations of MIR-G and 8-OH-MIR-G were determined by total MIR and total 8-OH-MIR (i.e., concentrations after hydrolysis) minus unconjugated MIR and unconjugated 8-OH-MIR, respectively. **RESULTS** Plasma levels of 8-OH-MIR were lower than those of MIR and DMIR (median 1.42 nmol/L vs. 92.71 nmol/L and 44.96 nmol/L, respectively). The plasma levels (median) of MIR-G and 8-OH-MIR-G were 75.00 nmol/L and 111.60 nmol/L, giving MIR-G/MIR and 8-OH-MIR-G/8-OH-MIR ratios of 0.92 and 59.50, respectively. Multiple regression analysis revealed that smoking was correlated with the plasma MIR concentration (dose- and body weight-corrected; $p=0.040$) and that age (years) was significantly correlated with the plasma DMIR concentration (dose- and body weight-corrected; $p=0.018$). **CONCLUSION** The plasma concentration of 8-OH-MIR was as low as 1.42 nmol/L, whereas 8-OH-MIR-G had an approximate 59.50-times higher concentration than 8-OH-MIR, suggesting a significant role for hydroxylation of MIR and its glucuronidation in the Japanese population.

1-YIA-07 YIA

Deficiency of cardiac natriuretic peptide signaling promotes peripartum cardiomyopathy-like remodeling in the mouse heart

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Background: The maternal circulatory system changes dynamically during perinatal period. Although atrial and brain natriuretic peptides (ANP and BNP, respectively) produced in the heart control circulatory homeostasis through their common receptor, NPR1, the pathophysiological roles of endogenous ANP/BNP in the perinatal period are not fully understood. ***Methods***: To clarify the pathophysiological roles of the endogenous ANP/BNP–NPR1 system during the perinatal period, the phenotype of female wild-type and conventional or tissue-specific Npr1-knockout mice during the perinatal period was examined. ***Results***: In wild-type mice, lactation but not pregnancy induced reversible cardiac hypertrophy accompanied by increases in fetal cardiac gene mRNAs and ERK1/2 phosphorylation. Npr1-knockout mice exhibited significantly higher plasma aldosterone level than did wild-type mice, severe cardiac hypertrophy accompanied by fibrosis, and left ventricular dysfunction in the lactation period. In the hearts of Npr1-knockout mice during or after the lactation period, an increase in interleukin-6 mRNA expression, phosphorylation of STAT3, and activation of the calcineurin–NFAT pathway were observed. Pharmacological inhibition of the mineralocorticoid receptor or neuron-specific deletion of the mineralocorticoid receptor gene significantly ameliorated cardiac hypertrophy in lactating Npr1-knockout mice. Anti–interleukin-6 receptor antibody administration tended to reduce cardiac hypertrophy in lactating Npr1-knockout mice. ***Conclusions***: These results suggest that the endogenous ANP/BNP–NPR1 system plays an important role in protecting the maternal heart from interleukin-6–induced inflammation and remodeling in the lactation period, a condition mimicking peripartum cardiomyopathy. (*Circulation,* in press)

1-YIA-08 YIA

2,5-Dimethylcelecoxib attenuates cardiac fibrosis after cryoinjury-induced myocardial infarction by suppressing the fibroblast-myofibroblast differentiation

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【Background】
Cardiac fibrosis is associated with heart diseases, such as myocardial infarction (MI), and activated fibroblasts (myofibroblasts) play a main role during fibrosis progression. Although we reported that 2,5 dimethylcelecoxib (DM-C) prevents cardiac fibrosis, the molecular mechanism, including the effect on myofibroblast differentiation, is not clarified yet.
【Objective】
We investigate the effect of DM-C on MI-caused fibrosis and fibroblast-myofibroblast differentiation using *in vivo* and *in vitro* models.
【Methods】
In vivo: Cryoinjury-induced MI (CMI) mouse model was employed. In DM-C group, the mice received DM-C for 4 weeks from 3 days before the operation. Cardiac function was evaluated with transthoracic echocardiography every week. Four weeks after operation, the heart was removed and the fibrosis area was evaluated.
In vitro: The effect DM-C on myofibroblast-differentiation induced by TGF-b using SD rat dermal fibroblast was examined.
【Results】
In DM-C group, the ejection fraction was increased than control group and, according with this, the fibrosis area is reduced. Further, DM-C significantly suppressed aSMA expression (myofibroblast marker) and the phosphorylation level of Akt, GSK-3b and Smad2/3.
【Conclusion】
These results suggest that DM-C attenuates cardiac fibrosis after MI through inhibition of fibroblast-myofibroblast differentiation. DM-C also inhibited the TGF-b /SMAD2/3 signaling pathway by Akt inhibition. Therefore, DM-C has a potential as the novel drug for treatment of cardiac fibrosis after MI.

1-YIA-09 YIA

Physiological role of gp130 receptor in newborn mouse cardiomyocyte development

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Mammalian ventricular cardiomyocytes (VCM) are still premature at birth and continue to proliferate and differentiate by maturating mitochondria and excitation-contraction coupling system for a certain period after birth. In the heart at this period, the concentration of various humoral factors changes. However, it is still unknown which of them are responsible for the VCM development. Here, we examined a role of gp130, a main subunit of receptors for the IL6-family of cytokines in this process. A specific gp130 inhibitor, SC144 (3 mg/kg) or its vehicle was subcutaneously injected to mice daily from day 1 to 20 after birth. SC144 significantly increased the heart and lung weights as compared with vehicle. Although SC144 did not cause arrhythmia, it induced a significant decrease in the contractility of the left ventricle (LV) with thinning of the LV wall without dilation of the inner diameter as assessed with echocardiogram and histological analysis. In isolated VCM, SC144 significantly shortened their longitudinal and disorganized T-tubular structure. SC144-treated VCM exhibited a significantly reduced peak amplitude of L-type Ca^{2+} channel currents and twitch Ca^{2+} transients compared with the control. Taken together, these results suggest that gp130 plays crucial roles in the VCM development in a mouse perinatal stage.

1-YIA-11 YIA

Therapeutic silencing of centromere protein X ameliorates hyperglycemia in zebra fish and mouse models of type 2 diabetes mellitus

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Type 2 diabetes mellitus (T2DM) is characterized by persistent hyperglycemia, an d contributed by genetic and environmental factors. Optimum T2DM management involves early diagnosis and effective glucose-lowering therapies. Further research is warranted to improve our understanding of T2DM pathophysiology and reveal potential roles of genetic predisposition. We have previously developed an obesity- induced diabetic zebrafish model that shares common pathological pathways with humans and may be used to identify putative pharmacological targets of diabetes. Additionally, we have previously identified several candidate genes with altered expression in T2DM zebrafish. Here, we performed a small-scale zebrafish screening for these genes and discovered a new therapeutic target, centromere protein X (CENPX), which was further validated in a T2DM mouse model. In zebrafish, cenpx knockdown by morpholino or knockout by CRISPR/Cas9 system ameliorated overfeeding-induced hyperglycemia and upregulated insulin level. In T2DM mice, small-interfering RNA-mediated Cenpx knockdown decreased hyperglycemia and upregulated insulin synthesis in the pancreas. Gene expression analysis revealed insulin, mechanistic target of rapamycin, leptin, and insulin-like growth factor 1 pathway activation following Cenpx silencing in pancreas tissues. Thus, CENPX inhibition exerted antidiabetic effects via increased insulin expression and related pathway s. Therefore, T2DM zebrafish may serve as a powerful tool in the discovery of new therapeutic gene targets.

1-YIA-12 YIA

SMTP-44D improves diabetic neuropathy in mice

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Diabetic neuropathy (DN) is one of the major complications of the diabetes and has a prevalence as high as 50 % of diabetic patients. However, there are few approved effective therapies against painful or insensate DN. In the present study, we evaluated the effect of SMTP-44D in mice model of DN. SMTP-44D (0.3, 3 and 30 mg/kg) was administered to 200 mg/kg streptozotocin (STZ)-induced diabetic mice from the 1st to the 4th weeks after the injection of STZ. The effect of SMTP-44D was evaluated by mechanical allodynia, thermal hyperalgesia, and velocity of conduction and blood flow of sciatic nerve. Furthermore, levels of inflammatory cytokines and oxidative stress in sciatic nerve by administration of SMTP-44D were assessed by ELISA and TBARS assay, respectively. To assess neurological degeneration, the G-ratio and the myelin thickness of Schwann cells in the sciatic nerve were measured. The treatment with SMTP-44D dose-dependently ameliorated allodynia, hyperalgesia, and velocity of conduction and blood flow of sciatic nerve in diabetic mice of DN. Furthermore, levels of inflammatory factors were also improved. These results indicate that SMTP-44D shows potential as a new therapeutic agent for DN.

1-YIA-13 YIA

Differentiation-inducing factor-1 suppresses breast cancer cell proliferation by reducing STAT3-mediated cyclin D1 expression

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Differentiation-inducing factor-1 (DIF-1) identified from *Dictyostelium* inhibits the proliferation of various cancer cells. However, the precise mechanism of DIF-1's action remains undetermined. Here, we investigated whether DIF-1 prevents tumor growth *in vivo* and how DIF-1 inhibits cell proliferation using breast cancer MCF-7 and 4T1 cells.
First, we performed experiments using cancer model mice made by injecting the cells into mammary fat pad. Oral administration of DIF-1 significantly suppressed the primary tumor growth without adverse effects.
DIF-1 strongly suppressed the proliferation of MCF-7 and 4T1 cells reducing the expression levels of STAT3 and cyclin D1. S3I-201, a STAT3 inhibitor, and the siRNA for STAT3 reduced cyclin D1 and inhibited cell proliferation, indicating that the reduction of cyclin D1 was caused by the reduction of STAT3. In MCF-7 cells, DIF-1 did not reduce STAT3 mRNA or promote STAT3 protein degradation, suggesting that DIF-1 inhibited STAT3 protein synthesis. We revealed that DIF-1 inhibited the phosphorylation (activation) of $p70^{S6K}$/$p85^{S6K}$. Inhibition of $p70^{S6K}$/$p85^{S6K}$ using rapamycin, an mTOR inhibitor, also reduced the expressions of STAT3 and cyclin D1.
In conclusion, DIF-1 exhibits anti-proliferative effect by reducing STAT3-mediated cyclin D1 in breast cancer cells. The inhibition of STAT3 by DIF-1 was caused by the suppression of protein synthesis through the inhibition of $p70^{S6K}$/$p85^{S6K}$. Our findings suggest that a novel anti-cancer agent against breast cancer could be developed using DIF -1 as a lead compound.

1-YIA-14 YIA

Cancer cells co-opt the neuronal redox-sensing channel TRPA1 to promote oxidative-stress tolerance

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Generation of reactive oxygen species (ROS), a natural byproduct of oxygen metabolism, occurs in all aerobic organisms at a controlled rate. Cancer cells are subjected to numerous cellular insults, including dysregulated oncogenes and dissociation from their natural extracellular matrix niches, leading to the generation of high levels of ROS; therefore, defense system against oxidative stress is critical for cancer cell survival. Here, we show a non-canonical oxidative-stress defense mechanism through TRPA1, the redox-sensing Ca^{2+}-influx channel that we previously found in sensory and vagal neurons (Takahashi et al., *Nature Chem. Biol.*, 2011). In TRPA1-enriched breast and lung cancer spheroids, TRPA1 induces Ca^{2+} influx in response to ROS generated upon matrix deprivation in the inner spheroid cells and suppresses apoptosis, and its inhibition induces clearance of cells from the inner space. TRPA1 is also activated by ROS-inducing chemotherapies and drives chemoresistance, and its inhibition suppresses xenograft tumor growth and enhances chemosensitivity. TRPA1 does not affect cellular redox status but allows cancer cells to tolerate harsh oxidative stress through upregulation of Ca^{2+}-induced anti-apoptotic programs involving MCL-1. Interestingly, NRF2, an oxidant-defense transcription factor, directly controls TRPA1 expression, thus providing an orthogonal program for protection against oxidative stress together with canonical ROS-neutralizing programs that reduce oxidative stress. Thus, our study reveals a previously unrecognized oxidative-stress tolerance program involving a neuronal ROS sensor, TRPA1 and highlights its potential as a therapeutic target.

1-YIA-16 YIA

Whole-organ profiling of cancer metastasis by tissue clearing and light-sheet fluorescent microscopy

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It has been recognized that interactions between cancer cells and stroma are important for survival of cancer cells and metastasis at distant organs. In addition, the complexity within tumor microenvironment contributes to resistance of conventional therapy. Therefore, despite decades of cancer researches, it is still difficult to elucidate the complexity of tumor microenvironment at whole organ or whole body level. To understand tumor microenvironment and overcome cancer, it is necessary to detect and quantify sparsely distributed metastatic cells throughout the body or organ at single-cell resolution.

Here, we demonstrate that CUBIC (clear, unobstructed brain/body imaging cocktails and computational analysis)-based cancer (CUBIC-Cancer) analysis with refractive-indices (RI) optimized protocol enables comprehensive cancer cell profiling in whole body and organs. We applied CUBIC-Cancer analysis to a dozen mouse models using several cancer cells and spatio-temporal quantification of metastatic cancer progression at single-cell resolution. As a result, metastatic foci can be observed and quantified through whole organ or whole body at single-cell resolution. In addition, three-dimensional (3D) monitoring revealed that the patterns of metastasis were dependent upon cancer cell or metastatic organs. Comparing two cancer cell lines, we quantified the difference of metastatic processes between angiogenesis and vessel cooption. Whole-organ profiling with single-cell resolution also enables to quantify the early steps of lung metastasis formation and rejection.CUBIC-Cancer analysis suggests that the epithelial-mesenchymal transition promotes not only extravasation but also cell survival at metastatic sites. CUBIC-Cancer analysis is applicable to pharmacotherapeutic profiling of anti-tumor drugs. CUBIC-Cancer analysis is compatible with in vivo bioluminescence imaging and 2D histology.We suggest that a scalable analytical pipeline with these three modalities may contribute to addressing currently incurable metastatic diseases.

1-YIA-17 YIA

Critical moieties of aromatic amino acid probes causing renal accumulation in tumor imaging

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As molecular probes for cancer diagnosis, [^{18}F]FAMT (3-[^{18}F]fluoro-α-methyl-l-tyrosine) and [^{123}I]IMT (3-[^{123}I]iodo-α-methyl-l-tyrosine) have been used clinically due to their high selectivity to cancer-specific amino acid transporter LAT1 (SLC7A5). However, FAMT and IMT exhibit strong physiological background only in the kidney. Previous study shows FAMT is a substrate of organic ion transporters. Moreover, it has been confirmed that the renal excretion of the probes was inhibited by probenecid, so the organic anion transporter OAT1 (SLC22A6) that mediates urinary excretion of organic anions is supposed to be important for the renal accumulation of the probes. Here, we examined a series of aromatic amino acid derivatives with the altered positions for hydroxyl groups and halogen groups on the aromatic ring. By comparing their IC_{50}s to inhibit the uptake of *para*-aminohippurate by OAT1 and their efflux profiles, we revealed that both halogen and hydroxyl group on the benzene ring of FAMT and IMT are critical for the interaction, whereas the α-methyl moiety essential for the selectivity to LAT1 is not important to interact with OAT1. The results would benefit for the design of the tumor-specific imaging probes with less renal background and the radionuclide therapeutic agents with less adverse renal damage.

1-YIA-18 YIA

Rapid measurement of plasma concentration of a molecular-targeted agent, pazopanib, with diamond sensor.

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Molecular-targeted anticancer drugs elicit less toxicity than conventional reagents. Yet, patients often suffer from severe adverse effects. A reason is 'fixed dosage' administration of the drug to all the patients regardless of their body size and complications; because of this strategy, the plasma concentration seems to exceed the therapeutic window occasionally. Although frequent measurement of the drug level at a clinical site is a solution, currently available methods such as mass spectrometry are time and cost consuming. To overcome these shortcomings, in this study, we developed a procedure with an electrochemical sensor composed of a conductive diamond, which yields more stable reactions than conventional materials. When guinea-pig plasma mixed with pazopanib, a multi-kinase inhibitor, was tested, the sensor detected a clinically relevant concentration of 3 to 300 μM. Time and sample amount necessary for each series of the measurement was <1 min and 100 μL, respectively. The sensor was repeatedly usable with minimal impairment of the sensitivity, saving the cost for the assay. This rapid and easily-handed method may enable therapeutic drug monitoring and accelerate tailored medicine for cancer.

1-YIA-19 YIA

Analysis of interaction between dimerized receptor and β-arrestin

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[Background] G-protein coupled receptors (GPCR) are known to form a dimerized homomeric or heteromeric receptors. However, coupling between dimerized receptor and β-arrestin is not well understood. We previously found that vasopressin can modulate morphine tolerance in ventral medulla through V1b receptor. However, how V1b, μ opioid receptors and β-arrestin 2 are arranged is not known. Here, we employed three molecule BRET (bioluminescence resonance energy transfer), which occur between split luciferase and green fluorescent protein (GFP), to monitor association of a receptor-containing complex. [Method] V1b and μ opioid receptors were connected at their carboxyl-termini to each part of split luciferase. β-arrestin 2 was connected to an enhanced green fluorescent protein. HEK cell were transfected with genes for receptors and β-arrestin 2. After agonist stimulation, BRET signal was measured by a plate reader. [Results] Receptors connected with split luciferase were functional in term of generating their cellular responses. V1b and μ opioid receptors formed homomeric or heteromeric receptor dimers, which were detect through luciferase intensities. Significant BRET signal was generated by interaction between receptor dimer and β-arrestin 2. [Conclusions] Our data suggested that the three-molecule BRET analysis can be applied to study interaction between receptor dimer and β-arrestin.

1-YIA-20 YIA

Identification of substrates in the brain of OCTN1/SLC22A4 based on untargeted metabolomics

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SLC22A4, also known as carnitine/organic cation transporter OCTN1 is ubiquitously expressed in the body. OCTN1 is functionally expressed in neurons, microglia, and neural stem cells in the brain, and may play a protective role in pathophysiological conditions via its *in vivo* substrate ergothioneine, a food-derived antioxidant. On the other hand, we have recently noticed that pentylenetetrazole-induced seizure was limitedly observed in *octn1* gene knockout (*octn1*$^{-/-}$) mice, and this may not be explained by the absence of this antioxidant. To find new OCTN1 substrates in the brain, we here conducted untargeted metabolome analysis using LC-TOF-MS. Hippocampus, cerebral cortex, and plasma of wild-type and *octn1*$^{-/-}$ mice were subjected to metabolomics, and 2,599, 2,676, and 1,697 ion peaks, respectively, were observed. Among them, five ion peaks with m/z 455, 426, 158, 154, and 144 exhibited at least two times difference between the two strains, only m/z 158 being found to be commonly lower in *octn1*$^{-/-}$ in all the samples. Time-dependent and saturable transport of chemically synthesized compound corresponding to this peak was observed in HEK293/OCTN1 cells. In addition, systemic elimination of this compound in *octn1*$^{-/-}$ mice was more rapid compared with wild-type. Thus, we have newly identified OCTN1 substrate in the brain.

1-YIA-21 YIA

Repagermanium attenuates H_2S-induced acceleration of $Ca_v3.2$ T-type calcium channel activity and pain sensitivity by directly interacting with H_2S

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Repagermanium, once hydrolyzed into THGP (3-(trihydroxygermyl)propanoic acid) in an aqueous solution, exhibits various biological activities and attenuates osteoporosis, pain, inflammation, etc., although the underlying molecular mechanisms remain unclear. The present study was conducted to see whether THGP would directly interact with H_2S, a gasotransmitter, generated by some enzymes including cystathionine-γ-lyase (CSE), which promotes pain sensation by increasing $Ca_v3.2$ T-type calcium channel (T-channel) activity. ^{1}H-NMR and LC-MS/MS spectrum analyses indicated that THGP reacts with SH^- derived from H_2S donors, NaSH or Na_2S, generating a sulfur-containing compound. In $Ca_v3.2$-transfected HEK293 cells, THGP abolished Na_2S-induced enhancement of T-currents. In mice, THGP suppressed the mechanical allodynia caused by intraplantar Na_2S or burn injury, as assessed by von Frey test, as did a T-channel blocker and CSE inhibitor. Western blotting demonstrated the burn injury-induced upregulation of CSE protein in the plantar skin. These data suggest that THGP directly interacts with H_2S, thereby attenuating H_2S-dependent enhancement of $Ca_v3.2$ activity and pain sensitivity. The burn injury-induced allodynia is considered to involve the CSE upregulation followed by acceleration of the $H_2S/Ca_v3.2$ pathway.

1-YIA-22 YIA

Comparison of two downstream signaling pathways in the μ-opioid receptors activated by several opioids

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In Japan, morphine (MRP), fentanyl (FEN), oxycodone (OXY) and hydromorphone (HDM) have been approved as pain analgesics in palliative and supportive care. Although all of them are selective for μ-opioid receptors (μOR), their receptor-activated signaling properties are different. Moreover, the combined and switching effets among them are complicated. Recent molecular analyses of the properties of μOR revealed two downstream pathways either eliciting analgesic effects through a G protein-mediated pathway or through the β-arrestin-mediated pathway that causes adverse events (AEs). However, molecular charactrerization of interaction among opioids has not investigated sufficiently. We therefore investigated characterization of these opioids using cells stably expressing μOR by three cell-based CellKey™, GloSensor™ cAMP and internalization assays.

To Detect μOR-mediated G protein-mediated signaling, both CellKey™ assay and GloSensor™ cAMP assay were used; the former is an electric impedance-based measurement, the latter is the measurement of the real-time cAMP levels. Among four opioids, the rank order of EC_{50} of each opioid was FEN≤HDM<MRP≤OXY in the above two assays. On the other hand, internalization assay suggested that internalization of μOR by activation of β-arrstin-mediated pathway occurred only in the case of FEN.

Based on these data got by single opioid, we are now on the way to investigate charactrestics of these pathways by simultaneous administration of several combinations of opioids.

1-YIA-23 YIA

GPR143, a L-DOPA receptor, is involved in monocrotaline-induced pulmonary hypertension in rats

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We previously demonstrated that L-DOPA modulated the vascular α1-adrenergic receptor through GPR143, a G-protein coupled receptor, and sensitized vasomotor tone. The purpose of this study is to clarify the involvement of GPR143, in pulmonary hypertension (PH). We generated GPR143 gene-deficient (KO) rats and comparatively studied monocrotaline (MCT) -induced PH in wild type (WT) and *Gpr143*-KO rats. We evaluated the interaction between L-DOPA and adrenergic α1 receptor by contractile force of rat isolated pulmonary arteries. The degree of PH was evaluated by right ventricular systolic pressure (RVSP) and right ventricular to body weight ratio (RV/BW). In isolated pulmonary arteries, L-DOPA (1 μM) augmented contractile response to phenylephrine, an α1 adrenergic receptor agonist. One month after injection subcutaneously with MCT (60 mg/kg), the RVSP was attenuated in *Gpr143*-KO rats as compared to the WT rats (49.7 +/- 1.1 mmHg and 41.1 +/- 1.4 mmHg in WT and *Gpr143*-KO, $p<0.01$, N=5). Coordinately, the RV/BW was also reduced in *Gpr143*-KO rats compared to the WT rats ($5.8 +/- 0.3 \times 10^{-4}$ and $4.9 +/- 0.2 \times 10^{-4}$ in WT and *Gpr143*-KO, $p<0.05$, N=7). We here provide evidence that GPR143 is involved in MCT-induced PH in rats. Further studies are needed to elucidate detailed mechanisms.

1-YIA-24 YIA

Establishment of a new method for long-term single-molecule fluorescence imaging and its application to synaptic molecules

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Single-molecule fluorescence imaging (SMFI) is a promising method to unvail dynamic features of molecules underlying physiological and pharmacological responses of cells. However, the time window for SMFI is limited due to photobleaching of the labeled dye, precluding detailed analysis. Here we aim to overcome the limitation of SMFI by developing a protein tag system named DeQODE tag system, consisting of two components: Quenched Organic Dye Emission (QODE) probe, a small organic dye coupled with a quencher moiety, and DeQODE tag, a single-chain antibody that turns on fluorescence emission of the QODE probe by binding the quencher. We expected that reversible and repeatable cycles of association and dissociation between QODE probe and DeQODE tag enable virtually perpetuating fluorescent tagging of the target protein. We tested the applicability of DeQODE tag system to SMFI in COS7 cells expressing DeQODE tag-fused syntaxin 1A and a synaptic protein Munc13-1. Fluorescent spots of QODE probe repeatedly appeared and disappeared for at least an hour. Also, the decrease in syntaxin 1A mobility was detected upon the application of phorbol ester recruiting Munc13-1 to plasma membrane. These results show that the DeQODE tag system-based long-term SMFI enables detailed analysis of molecular dynamics of signaling proteins in living cells.

1-YIA-25 YIA

Auxin-mediated rapid degradation of target proteins in neurons

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Genetic manipulation of protein levels is a promising approach to identify the function of a specific protein in living organisms. Previous studies demonstrated that the auxin-inducible degron strategy provides rapid and reversible degradation of various proteins in fungi and mammalian mitotic cells. In this study, we employed this technology to postmitotic neurons to address whether the auxin-inducible degron system could be applied to the nervous system. Using adeno-associated viruses, we simultaneously introduced enhanced green fluorescent protein fused with an auxin-inducible degron tag and an F-box family protein, TIR1 from Oryza sativa (OsTIR1), into hippocampal neurons from mice. In dissociated hippocampal neurons, enhanced green fluorescent protein fluorescence signals rapidly decreased when adding a plant hormone, auxin. Furthermore, auxininduced enhanced green fluorescent protein degradation was also observed in hippocampal acute slices. Taken together, these results open the door for neuroscientists.

1-YIA-26 YIA

Ph□osphorylation of Npas4 by MAPK regulates reward-related gene expression and behaviors

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Dopamine (DA) activates MAPK via PKA/Rap1 in medium spiny neurons (MSNs) expressing the dopamine D1 receptor (D1R)in the nucleus accumbens (NAc), thereby regulating reward-related behavior.However, howMAPKregulates reward-relatedlearning and memory through gene expression is poorly understood. Here, to identify the relevant transcriptional factors, we performed proteomic analysis using affinity beads coated with CREB-binding protein (CBP), a transcriptional coactivator involved in reward-related behavior. We identified more than 400 CBP-interacting proteins, including Neuronal Per Arnt Sim domain protein 4 (Npas4). We found that MAPK phosphorylated Npas4 downstream of PKA, increasing the Npas4-CBP interaction and the transcriptional activity of Npas4 at the brain-derived neurotrophic factor (BDNF) promoter. The deletion of Npas4 in D1R-expressing MSNs impaired cocaine-induced place preference, which was rescued by Npas4-WT but not by a phospho-deficient Npas4 mutant. These observations suggest that MAPK phosphorylates Npas4 in D1R-MSNs and increases transcriptional activity to enhance reward-related learning and memory. (Funahashi et al., Cell Reports, 2019)

1-YIA-27 YIA

In vivo whole-cell recordings from amygdalar neurons

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The basolateral amygdala (BLA) is a deep brain region that contributes to emotional processing and may be activated together with the prefrontal cortex. For example, the BLA and the dorsomedial prefrontal cortex are synchronized at 4-Hz during fear behavior. However, the neural basis of the synchronization has not been addressed, mainly because it is difficult to identify projecting neurons using extracellular recordings, which are widely used to record neuronal firing activity. Patch-clamp recordings, an intracellular recording technique, enable to record synaptic inputs and label the projection of the recorded neurons. However, there are few reports using in vivo whole-cell recordings from the BLA because impurities on pipette tips make it difficult to attain gigaseal, a critical step for the whole-cell configuration. In this study, we have developed a new method to achieve whole-cell recording from in vivo mice deep brain regions. We elaborated a double tube system; that is, a guide cannula for patch-clamp pipettes through which a tissue-boring stick is inserted in advance. Using them, we more easily achieved in vivo whole-cell recordings from deep brain regions because the pass length for which the pipettes needed to go through the brain parenchyma were reduced. We will present examples of recordings from BLA neurons which is located about 4 mm deep from the brain surface, together with recordings of local field potentials from the medial prefrontal cortex.

1-YIA-28 YIA

Optogenetic recording and manipulation of median raphe serotonin neurons in reward-related behavior.

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Reward processing is an essential function for survival of individuals. Impairment in reward processing induces an objectively incorrect judgment about the value of reward, leading to drug addiction or anhedonia. Increasing evidences implicate that serotonergic neurons as well as dopaminergic neurons play a critical role in the reward processing. Median raphe nucleus (MRN), containing lots of serotonergic neurons, is suggested to be involved in this process. However, it is not fully understood whether MRN serotonergic neurons respond to reward and how they regulate reward-related behavior, partly because of the difficulty of specific recording and manipulation of MRN serotonergic neurons. Here, we determined whether and how MRN serotonergic neurons control reward system and reward-related behavior by optogenetic recording and manipulation. Fluorescent calcium indicator, GCaMP6s, was expressed in MRN serotonergic neurons, by serotonergic neuron specific viral vectors. Then we measured the fluorescence in the MRN before and after sucrose licking by using fiber photometry. We found that the fluorescence started to decrease just after sucrose licking, indicating that reward inhibits MRN serotonergic neurons. Moreover, optogenetic inactivation of MRN serotonergic neurons increased the number of nose-poke and spent time in the chamber associated with stimulation in self-stimulation paradigm and conditioned place preference paradigm, respectively. Our results suggest the MRN serotonergic neurons play a key role in the reward processing.

1-YIA-29 YIA

Neural mechanisms underlying regulation of motivation for voluntary wheel running in mice

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It is well known that mice rotate running wheels (RWs) persistently without any rewards, reminiscent of behavioral addiction. In the present study, by focusing on this characteristic feature of mice, we investigated neural mechanisms that regulate the motivation for rotating RWs. Individual male C57BL/6J mice were trained to rotate RWs until the number of rotations became stable by giving free access to a RW for 30 min every other day. Subcutaneous injection of a 5-HT_{1A} receptor (5-HT_{1A}R) antagonist WAY100635 (WAY, 1 mg/kg) significantly increased the number of rotations, while a 5-HT_{2A}R antagonist volinanserin (Vol, 0.01 mg/kg) or a 5-HT_{2C}R antagonist SB242084 (SB, 0.3 mg/kg) reduced it. To investigate whether the changes in rotation are caused by changes in locomotor activity, open field test was performed. Although WAY and Vol did not affect locomotor activity, SB increased locomotor activity. These results suggest that the WAY-, Vol-, or SB-induced changes in rotation number of RWs may not be accounted for by the changes in locomotor activity. Taken together, present findings imply that 5-HT_{1A}R-, 5-HT_{2A}R-, and 5-HT_{2C}R-mediated transmission may differently regulate the motivation for rotating RWs in mice and that the latter two might be involved in enhancing the motivation for behavioral addiction.