

Keynote Lecture

Novel Neuronal-Glial Signaling in Magnocellular and Parvocellular Neurons of the Hypothalamic Paraventricular Nucleus

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It is increasingly clear that glial cells can no longer be relegated to the role of supporting cast members of the central nervous system, and that they perform critical signaling functions that contribute to signal transmission in neural circuits. The notion of the tripartite synapse, according to which signal transmission occurs among pre- and postsynaptic neural elements and astrocytic partners, is now well accepted. However, this neuronal-glial signaling is highly localized to the synapse, where intercommunication between astrocytes and neurons modulates presynaptic release probability and postsynaptic responses. We recently discovered a novel form of neuronal-glial signaling in the hypothalamic paraventricular nucleus (PVN) that exploits the full spatial domain of astrocytes to transmit retrograde signals to distal upstream neuronal targets. Thus, vasopressin and corticotropin releasing hormone (CRH) neurons in the PVN transmit retrograde signals to distal glutamate and/or GABA neurons via dendritic neurotransmitter/neuropeptide release and activation of an astrocyte signaling intermediate. The upstream glutamate and GABA neurons project back to the vasopressin and CRH neurons to form a closed neuronal-glial-neuronal signaling loop. This novel form of retrograde trans-neuronal-glial transmission allows PVN neurons to regulate their synaptic inputs by controlling presynaptic neuron firing, thus providing a powerful means of influencing their hormonal output. This work was supported by NIH grants NS042081 and MH066958, and by the Pierson Chair in Neuroscience.



Jeffrey G. Tasker, Ph.D.

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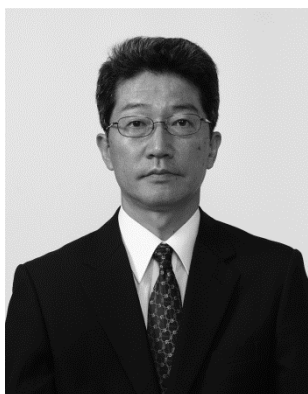
Jeffrey Tasker received his bachelor's degree in biology from the University of Colorado in 1981 and his doctorate in neuroscience from the University of Bordeaux, France in 1986. He received postdoctoral training in the Physiology Dept at the Tulane University Health Science Center and in the UCLA Neuropsychiatric Institute. He joined the faculty of Tulane University as an assistant professor in the Department of Cell and Molecular Biology in 1991, and currently holds the rank of professor. He has had continuous research funding from the National Institutes of Health for over 20 years and has published over 75 research papers, reviews and book chapters. He became the Catherine and Hunter Pierson Chair in Neuroscience in 2005, served as director of the Tulane University Neuroscience Program from 2006 to 2014, and is currently director of the Division of Neurobiology of the Cell and Molecular Biology Department. He has served on several NIH and NSF grant review panels and on the editorial board of the journals *Endocrinology*, *Stress*, and *Steroids*. The research in his laboratory uses electrophysiology and molecular biology approaches to study the electrical activity and molecular signaling of neuroendocrine cells of the hypothalamus and neurons of the amygdala, with implications for the understanding and future treatment of such central nervous system conditions as stress, depression, obesity, and posttraumatic stress disorder.

Formation of Endoplasmic Reticulum-Associated Compartment (ERAC) in Vasopressin Neurons: A Mechanism by Which ER Stress Is Reduced

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Analyses of a mouse model for familial neurohypophysial diabetes insipidus (FNDI), characterized by progressive polyuria due to progressive decreases in arginine vasopressin (AVP) release, revealed that mutant proteins are accumulated in a sub-compartment of endoplasmic reticulum of AVP neurons. By forming such a structure called ER-associated compartment (ERAC), AVP neurons are likely to reduce ER stress. However, the formation of ERAC is hampered in relatively old FNDI mice or in the mice subjected to chronic dehydration. Failure of ERAC formation induces autophagy in the AVP neurons, which finally die through the mechanism called autophagy-associated cell death. ATF6 α , one of three ER stress sensors, seems to contribute to the formation of ERAC through the upregulation of ER chaperone such as BiP. It is also of note that, while enlargement of a sub-compartment of ER, a similar structure to ERAC, was observed in the AVP neurons in wild-type mice subjected to dehydration, dilation of ER was diffused in the AVP neurons in dehydrated ATF6 α knockout mice. Thus, our data suggest that misfolded proteins are confined to the ERAC in the AVP neurons of not only FNDI but also wild-type mice, in order to reduce ER stress.



Hiroshi Arima, M.D., Ph.D.

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Hiroshi Arima is a graduate of Nagoya University in 1988. After the M.D. degree, he obtained his Ph.D. degree from Nagoya University in 1997. Following an appointment as a clinical and research fellow at Nagoya University, he worked at Section on Endocrine Physiology, Developmental Endocrinology Branch, National Institute of Child Health and Human Development, National Institutes of Health, as a visiting fellow from 1998 through 2001. After coming home, he was appointed a clinical and research associate, assistant professor and then associate professor at Nagoya University. He became Professor of Endocrinology and Diabetes at Nagoya University Graduate School of Medicine in 2015. He is a clinical endocrinologist and specialized in water and electrolytes balance as well as energy balance of an organism.

Study of the Molecular and Physiological Mechanism Regulating Social Memory by Neurons Expressing Oxytocin Receptor

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Interest to neurohypophysial hormone oxytocin (OXT), basically synthesized in PVN magnocellular neurons and parvocellular neurons and SON, is now quickly growing with its curing effect, when administered to ASD patients, as a new pharmaceutical to improve social behaviors of them. We generated oxytocin gene deficient and oxytocin receptor gene deficient mice lines, observed impaired social behaviors with them, and considered these line as a mice-level ASD model animal. We are now trying to clarify the mechanism, how deletion of oxytocin receptor gene leads to the onset of abnormal social behaviors, and the localization of neurons expressing oxytocin receptor, of which activation gives rise to normalization of social behaviors in mice. With this strategy, we design experiments to elucidate the pharmaceutical mechanism by which the administration of OXT has a therapeutic effect on ASD patients. In the analysis of the brain prepared from *oxtr*-venous knock-in mice, we found that various nuclei related to social behaviors expressed OXTR in different type neurons, such as serotonergic, GABAergic, Glutamatergic and dopaminergic in nuclei-specific manners. OXTR-expressing neurons in medial amygdaloid nucleus (MeA) and lateral septal nucleus (LS) might have a higher contribution on the regulation of social memory. On the other hand, higher population of OXTR-expressing neurons in MeA (30%) and in LS (~100%) were characterized to be GABAergic and calretinin(+) interneurons. With the hypothesis that the major population of neurons expressing OXTR were GABAergic, we generate GABAergic neuron-specific OXTR gene KO mice, by crossing *Vgat-IRES-Cre* line with *Oxtr(fx/fx)* line. Resultant mice showed impaired social novelty. We continuously analyzed the responsible neurons expressing OXTR on social behaviors and especially social novelty, in LS and MeA.



Katsuhiko Nishimori, Ph.D.

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Katsuhiko Nishimori is a graduate of University of Tokyo in 1977, and he also obtained his Ph.D. degree from University of Tokyo in 1982. After working as a post-doc at Dr. Teruhiro Beppu's lab at University of Tokyo, he became an assistant professor at Laboratory of Enzymology, Institute of Applied Microbiology, University of Tokyo. Then, he was appointed as associate professor at Graduate Program in Agricultural Science, Tohoku University. He worked as a visiting associate professor at Department of Pathology, Baylor College of Medicine at Houston from 1994 through 1996. He participated in generating the oxytocin knockout mouse during his stay at Baylor. After coming back to Sendai, he was appointed Professor of Molecular Biology in 2001 at Graduate Program in Agricultural Science, Tohoku University. He developed many novel mouse lines, and among them, is the oxytocin receptor knockout mouse which is of great value in elucidating the mechanism of oxytocin.

Neural, Hormonal and Experiential Control of Social and Emotional Behavior

Sonoko Ogawa

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We have been studying brain mechanisms of social behavior, particularly regulation of sex-specific sexual and aggressive behavior by gonad steroids. In our recent studies, we also have focused on social interactive behaviors including social preference, social recognition and social memory as well as emotional and anxiety-related behavior in social context. Behavioral analysis in knockout mice of two types of estrogen receptors, ER α or ER β , revealed that ER β might be involved in 'modulatory' regulation of various social and emotional behaviors. Considering differential localization between two types of ERs, we are currently testing several hypotheses that ER β in the paraventricular nucleus of hypothalamus, medial amygdala, dorsal raphe nuclei, and locus coeruleus may mediate genomic action of estradiol. In this talk, we will overview our recent findings from behavioral analysis in mice with brain site-specific manipulation of ER gene expression and discuss possible neural, hormonal and experiential control of social and emotional behavior.



Sonoko Ogawa, Ph.D.

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Sonoko Ogawa is a graduate of the University of Tsukuba in 1979, and she also obtained her M.A. degree at the same university. She obtained the Ph.D. degree at the University of Connecticut in 1988. She moved to the Rockefeller University, New York, and was appointed a postdoctoral associate, research associate, assistant professor, and then associate professor in the Laboratory of Neurobiology and Behavior. In 2004, she became a full professor in the Laboratory of Behavioral Neuroendocrinology at the University of Tsukuba. She is also a visiting professor in the School of Medicine and Health Sciences at Monash University Sunway Campus, Malaysia, as well as an adjunct faculty member in the Laboratory of Neuroendocrinology at the Rockefeller University. She is specialized in behavioral neuroendocrinology and has been studying the brain mechanisms of social and emotional behaviors. She is an awardee of Young Psychologist Award at the 24th International Congress of Psychology in 1988, UConn Alumnae Who Have Made a Difference by the University of Connecticut Alumni Association in 1988, the Rockefeller University/Karolinska Institute Nicholson Award, 2003, and University of Tsukuba, Academic Year 2012 Best Faculty Member Award in 2013.

Noxious or Non-Noxious Inputs to Oxytocin Neurons: Possible Roles in the Control of Behaviors

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Oxytocin plays an essential role in milk ejection and parturition in mammals. Oxytocin has also been shown to be involved in the control of various behaviors, including anxiety-related behaviors, eating behaviors and affiliative behaviors. We have previously shown that stressful stimuli such as noxious stimuli and conditioned fear stimuli, activate hypothalamic oxytocin neurons via activation of brainstem catecholaminergic/prolactin-releasing peptide (PrRP)-positive neurons. Oxytocin neurons are activated not only by aversive stimuli but also possibly by pleasant stimuli. Food intake has been shown to induce activation of oxytocin neurons. We found that PrRP neurons are also involved in activation of oxytocin neurons following food intake. Social contact may also affect activity of oxytocin neurons. Non-noxious pleasant tactile stimuli induced 50-kHz ultrasonic vocalization, an index of positive states in rats, and activated hypothalamic oxytocin neurons. Physiological roles of oxytocin released during stressful or pleasant stimuli remain to be clarified. However, application of oxytocin has been shown to have anxiolytic, anorexic and pro-social actions. In fact, we have shown that endogenous oxytocin reduces anxiety-related behaviors, decreases amounts of food intake per meal and facilitates social recognition via various neural pathways.



Tatsushi Onaka, M.D., Ph.D.

Professor of Physiology, Jichi Medical University

Tatsushi Onaka obtained his M.D. degree from the University of Tokyo in 1985, and his Ph.D. degree from Jichi Medical University in 1990. He became an assistant professor at Jichi Medical University, and then worked at Babraham Institute, Cambridge, UK, as a British Council Fellow from 1992 through 1994. After coming home, he was appointed an associate professor, and in 2006, he became Professor of Physiology at Jichi Medical University. He is specialized in the physiological and endocrinological regulatory mechanisms of neuroendocrine peptides as well as their implications in animal behavior. Among other neuroendocrine peptides, he is especially interested in oxytocin and prolactin-releasing peptide. He is an awardee of Kawakami Prize of Japan Neuroendocrine Society in 2003. He gave a Mortyn Jones Memorial Lecture of British Neuroendocrine Society in 2011.

Optogenetic Approach to Regulate Neuronal Activity in Rat Vasopressin Neuron

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Optogenetic approach is a powerful tool to regulate neuronal activity by light-sensitive ion channels and investigate the interaction between neuronal network and behavioral change. Here, we have generated a transgenic rat that expresses the arginine vasopressin (AVP)-channelrhodopsin 2 (ChR2)-eGFP fusion gene. The eGFP that indicates the expression of the ChR2 gene was observed in the supraoptic nucleus (SON), the magnocellular divisions of the paraventricular nucleus (PVN) and the suprachiasmatic nucleus (SCN) that are known to localize AVP-containing neurons. Confocal laser scanning microscopic observation revealed that ChR2-eGFP was mainly localized in the membrane of magnocellular neurosecretory cells (MNCs) in the SON and the PVN of transgenic rats. The eGFP was also observed in the fibers originated from AVP neurons and posterior pituitary gland. The intensities of eGFP in those nuclei were markedly increased after chronic salt loading (2% NaCl to drink for 5 days). Using whole cell patch-clamp recordings in *in vitro* preparations such as a single cell isolated from the SON and brain slice including the SON of AVP-ChR2-eGFP transgenic rats, repeated blue light evoked action potentials repetitively in a current clamp mode, and caused inward currents in a voltage clamp mode. Optogenetic manipulations to regulate neuronal activity of AVP MNCs have come true, using transgenic techniques.



Yoichi Ueta, M.D., Ph.D.

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Yoichi Ueta is a graduate of University of Occupational and Environmental Health, from which he obtained his M.D. degree in 1987, and his Ph.D. degree in 1991. Then he worked at Babraham Institute (Dr. Gareth Leng), Cambridge, UK, as a research fellow. After coming home, he was appointed an instructor at University of Occupational and Environmental Health. He worked at Department of Medicine (Prof. Stafford Lightman), University of Bristol, as a visiting researcher from 1993 through 1995. After serving as a lecturer, he became Professor of Physiology, University of Occupational and Environmental Health in 2000. He is specialized in the physiological mechanisms of neurohypophysial hormones and neuropeptides related to stress response. Recently, he developed rat lines whose vasopressinergic or oxytocinergic neurons are labeled by fluorescent proteins. He is an awardee of Kawakami Prize of Japan Neuroendocrine Society in 1998.

Characterization and Future Perspectives of the Corticotropin-Releasing Factor-Modified Yellow Fluorescent Protein-Knock-In Mouse

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Corticotropin-releasing factor (CRF) produced in the paraventricular nucleus of the hypothalamus (PVH) plays a pivotal role in the regulation of the hypothalamic-pituitary-adrenal (HPA)-axis. All stress inputs are conveyed to hypothalamic CRF neurons via neural pathways and humoral factors. It has not been fully understood how CRF neurons are regulated by these inputs because experimental animals in which CRF neurons can be visualized with fluorescent proteins have not been available until recently. We developed a mouse line in which Venus (modified yellow fluorescent protein) gene was inserted into the CRF gene in frame by homologous recombination (*CRF-Venus*). Although Venus was colocalized with CRF in the PVH in the *CRF-Venus* mouse, a considerable discrepancy was observed. By bilateral adrenalectomy, most Venus-expressing neurons became expressing CRF, so the discrepancy may partly be explained by the suppressive glucocorticoid effect on the CRF promoter. Another mouse line was generated by deletions of the pgk-Neo cassette from the *CRF-Venus* genome (*CRF-Venus Δ Neo*); Venus fluorescence became more prominent, and most CRF neurons expressed Venus in physiological glucocorticoid states in the *CRF-Venus Δ Neo*. Most Venus-expressing neurons also expressed CRF mRNA in brain regions within and outside the PVH. Direct neural inputs to the Venus neurons, containing glutamate or γ -aminobutyric acid (GABA), were demonstrated by patch-clamp recordings. Effects of various transmitters on the glutamatergic or GABAergic inputs to the CRF neurons are now under investigation. With the same strategy for generating the *CRF-Venus*, *CRF-iCre* knock-in mouse was generated; it contains iCre sequence in frame instead of Venus. The iCre recombination took place selectively in brain regions that express CRF. *CRF-Venus*, *CRF-Venus Δ Neo*, and *CRF-iCre* will be useful tools for studying the regulatory mechanisms of CRF neurons by cellular and physiological viewpoints.



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Keiichi Itoi is a graduate of Tohoku University in 1980. After obtaining his Ph.D. degree from Tohoku University in 1987, he became a post-doc at Department of Pharmacology (Prof. Thomas Unger), University of Heidelberg, Germany, and next year, he was appointed an assistant professor at the same institute. After coming back to Japan in 1990, he became an assistant professor at Tohoku University Hospital, and later, an associate professor. He worked at Mental Health Research Institute, University of Michigan (Co-directed by Stan Watson and Huda Akil), from 1996 through 1998 as a visiting scholar. In 2001, he was appointed Professor of Information Biology at Graduate School of Information Sciences, Tohoku University, and also Professor of Neuroendocrinology at Graduate School of Medicine. He is an awardee of Kawakami Prize of Japan Neuroendocrine Society in 1995.

'Volume Transmission' Role of Oxytocin in the Regulation of the Male Sexual Function in the Spinal Cord

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A novel concept is proposed that certain neurons in the central nervous system may use both synaptic (or wiring) transmission and non-synaptic (or volume) transmission communications. We previously demonstrated that the gastrin-releasing peptide (GRP) system mediates spinal centers promoting penile reflexes in rats. A group of oxytocin neurons situated in the parvocellular part of the paraventricular nucleus (pPVN) and projecting to the spinal cord controls penile reflexes. Therefore, the hypothesis that oxytocin, which is transported by long descending paraventriculospinal pathways, activates proerectile spinal centers has been proposed. Subsequently, we have found that the axonal distribution of oxytocin in the lumbar spinal cord exhibits a male-dominant sexual dimorphism in rats. Furthermore, oxytocin binding and expression of the specific oxytocin receptor were both observed in the spinal GRP neurons. Consequently, oxytocin efferents might secrete oxytocin from the spinal axonal terminals, and regulate male sexual function through an oxytocin receptor-mediated mechanism in spinal GRP neurons. However, it has been reported that there are few functional synaptic contacts between oxytocin neurons in the pPVN and spinal GRP neurons, because most pPVN neurons remained unlabeled by retrograde trans-synaptic tracing with pseudorabies virus from the bulbocavernosus muscle in experiments performed to identify neural circuits controlling male sexual functions. Taken together, these results suggest that hypothalamic oxytocin projections in the lumbar spinal cord may function, at least partly, via a non-synaptic mechanism: a volume transmission.



Hirotsuka Sakamoto, Ph.D.

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Hirotsuka Sakamoto is a graduate of Hiroshima University in 1997. After obtaining the M.A. degree (neuroscience) in 1999, he also obtained his Ph.D. degree (neuroscience) in 2002 from Hiroshima University. He also obtained another Ph.D. degree (medical science) from Kyoto Prefectural University of Medicine in 2009. After a JSPS research fellow and post-doctoral fellow, he was appointed a research associate at Kyoto Prefectural University of Medicine. In 2009, he became Associate Professor of Natural Science at Ushimado Marine Institute, Graduate School of Natural Science and Technology, Okayama University. His research interests are sexual differentiation of the brain and spinal cord, genomic and non-genomic effects of hormones on neurons, and the effects of severe psychological stresses on neurons. He is a recipient of many awards for young investigators including those from Japan Neuroendocrine Society (2008), Japanese Association of Anatomists (2009), and Zoological Society of Japan (2012). He is an awardee of 2010 Young Scientists' Prize, the Commendation for Science and Technology from the Minister of Education, Culture, Sports, Science and Technology of Japan in 2010.

A New Hypothalamic Area Enriched with Perineuronal Nets Having Bidirectional Connections between the Lateral Septum

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The paraventricular hypothalamic nucleus (PVN) is a well-studied region as the center of regulating the hypothalamic-pituitary adrenal axis. The present study found a new in the anterior hypothalamus (AH) of mice, a triangular-shaped area between the PVN and the fornix. This area was clearly stained with the WFA lectin that is broadly used for labeling the specialized extracellular matrix structure called perineuronal nets (PNNs). WFA stained this region with a triangular morphology in coronal brain sections, we named this region perifornical area of the anterior hypothalamus (PeFAH). The aim of this study was to characterize the PeFAH in terms of its expressing neuropeptides, neural connections, and functions. Histological and DNA microarray analyses demonstrated that many of PeFAH neurons were non-GABAergic and highly expressed enkephalin (Enk) neuropeptides. Moreover, PeFAH neurons expressed calretinin, but not parvalbumin and calbindin. Neuronal tracing revealed that both enkephalin- and calretinin-expressing neurons in the PeFAH massively projected to the lateral septum. The PeFAH was received inputs from the lateral septum (LS) neurons expressing calbindin, indicating bidirectional neural connection between the PeFAH and LS. The c-Fos expression analysis indicated that activity of PeFAH neurons was increased by psychological stressors such as restraint, novelty, and aggression, but not induced by homeostatic stressors of dehydration and fasting. Considering neuronal subtype and projection, the region of a compact cluster of Enk-positive neurons within the PeFAH is comparable with the perifornical nucleus previously identified in rats.



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Mayumi Nishi is a graduate of Kyoto University, and obtained her bachelor's degree in pharmaceutical Science in 1980. She obtained her degree of M.D. from Kyoto Prefectural University of Medicine in 1991. She also obtained her Ph.D. degree from the same university in 1997. She worked at Department of Biology and Center for Neural Science, New York University at New York (Prof. Efrain C. Azmitia) as a visiting scientist from 1994 through 1996. After coming back to Japan, she was appointed an assistant professor, lecturer, and associate professor at Department of Anatomy and Neurobiology, Kyoto Prefectural University of Medicine.

In 2009, she became Professor of Anatomy and Cell Biology at Nara Medical University. Her major interest is the neuroendocrinology of stress and emotion and her research is aimed at understanding the mechanisms by which environmental factors interact with the genome to influence brain development and to produce diverse forms of neural plasticity over a lifetime. She is a 1999 awardee of Best Imaging Award of Japan Bioimaging Society.

Paraventricular Nucleus Mechanisms of Stress Integration

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Parvocellular paraventricular nucleus (PVN) neurons control activation of the hypothalamo-pituitary-adrenocortical (HPA) axis. These neurons synthesize and secrete corticotropin-releasing hormone (CRH), which is responsible for ACTH secretion and subsequently, glucocorticoid release. Our laboratory has used expression of Cre recombinase driven by a simple-minded-1 (Sim1) promoter to explore the impact of selective PVN (and SON)-targeted gene deletion on HPA axis activity. Use of Sim1 to delete the glucocorticoid receptor (GR) in PVN (and SON) neurons results in exaggerated ACTH and corticosterone responses to acute stress in male mice. In combination with electrophysiological data from the Tasker group, these results indicate that the canonical GR is required for fast feedback inhibition of the HPA axis. There was no effect of PVN GR deletion on responses to chronic variable stress exposure, indicating that the GR is not involved in limiting the impact of repeated stress exposure. To test mechanisms of PVN activation, we bred Sim1-Cre with glucagon-like peptide 1 receptor (GLP1R) flox mice to selectively block the actions of the putative stress-excitatory neuropeptide GLP1 on HPA activity. Deletion of GLP1R in PVN decreases both ACTH and corticosterone responses to stress and attenuates parvocellular PVN Fos activation, consistent with a role for PVN GLP1 in stress activation. Notably, Sim1-mediated PVN gene deletion also attenuated heart rate and blood pressure responses to acute stress, perhaps via reducing activation of PVN preautonomic neurons. Collectively, these data provide evidence for PVN GR and GLP1 in stress control, the former essential for stress inhibition, the latter for stress excitation.



James P. Herman, Ph.D.

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James P. Herman earned his B.S. in Chemistry/Psychology at Hobart College in 1979 and his Ph.D. from the University of Rochester in 1987, from the Department of Neurobiology and Anatomy. He worked as a post-doc at the Mental Health Research Institute, University of Michigan. He started his academic career in the Department of Anatomy and Neurobiology at the University of Kentucky, where he was an Associate Professor and the James and Barbara Holsinger Chair of Anatomy and Neurobiology. He joined the University of Cincinnati faculty in 2000 and is currently the Donald C. Harrison Professor and Vice-Chair for Basic Research in the Department of Psychiatry and Behavioral Neuroscience. He is also Director of the University of Cincinnati Neurobiology Research Center. He currently serves as Editor-in-Chief of *Stress*, as a section editor for the *European Journal of Neuroscience*, and on several editorial boards. Dr. Herman's major research interests include structural, functional and molecular biological principles underlying brain stress integration, with an emphasis on delineating mechanism linking stress with mental illness, neurodegeneration and metabolic disease. He has made major contributions to our understanding of the role of limbic neurocircuitry in stress adaptation and stress-related pathologies, and has applied to state-of-the-art approaches to delineate molecular mechanisms underlying stress hormone signaling in the brain.

Induction of Hypothalamic Neurons from Pluripotent Stem Cells

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Recently, various hypothalamic neurons have been successfully engineered from pluripotent stem cells including mouse and human embryonic stem cell lines. Because pluripotent cells have to follow the stepwise changes in the organogenesis, developmental analyses on hypothalamus have been inevitable that the numerous transcription factors determine specification, survival, and migration during the formation of specific neurons. Hypothalamic progenitor cells arise from ventricular zone at E10.5, which express Rax (retina and anterior neural fold homeobox) gene. Then Otp (orthopedia) and SF-1 (steroidogenic factor 1) respectively appear in the dorsal and ventral regions at E13.5, which subsequently produce various transcription factors required for final maturation of the hypothalamic neurons. In the pluripotent stem cells, rostral-dorsal hypothalamic-like progenitors (Rax⁺) are generated from floating aggregates in serum-free and chemically defined medium conditions with minimized exogenous patterning signaling. A certain population of the Rax⁺ progenitors eventually generate Otp⁺ neuronal precursor, and subsequently develop to various dorsal and lateral hypothalamic neurons including vasopressin, oxytocin, melanin-concentrating hormone, and orexin. The magnocellular vasopressinergic neurons release the hormone upon stimulation. On the other hand, treatment with sonic hedgehog on the floating culture induces differentiation markers including SF-1 that is specific for rostral-ventral hypothalamic-like precursors. These cells eventually produce NPY and POMC. Now it is possible to induce most types of the rostral-hypothalamic neuron from pluripotent stem cell, that the application of the cells would have advantages on the studies on specification, migration, drug development, and regenerative medicine.



Hiroshi Nagasaki, M.D., Ph.D.

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Hiroshi Nagasaki is a graduate of Nagoya University Medical School in 1989. He obtained his Ph.D. degree at Nagoya University in 1997. After working as a postdoctoral research fellow, he was appointed a research associate at Department of Pharmacology, University of California, Irvine (Dr. Civelli lab), in 2000. After coming back to Japan, he was appointed an assistant professor, and then associate professor at Department of Metabolic Medicine, Nagoya University, Graduate School of Medicine. He moved to Department of Physiology, Fujita Health University in 2011 and served as an associate professor. In 2015, he became Professor of Physiology, at Fujita Health University School of Medicine. He is specialized in the development of the hypothalamus, and he recently succeeded in differentiating pluripotent stem cells into specific classes of neuroendocrine neurons of the hypothalamus.

Transcriptional Regulation of Vasopressin Gene: Update in 2015

Yasumasa Iwasaki and Keiichi Itoi

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Expression of arginine vasopressin (AVP) in magnocellular neurons of the supraoptic/paraventricular nuclei of the hypothalamus is known to be regulated by osmotic stress. A part of the osmoregulation of AVP synthesis/release is under the control of osmoreceptors residing in the circumventricular organs (subfornical organ and organum vasculosum of the lamina terminalis), and the molecular mechanism of osmosensation has been unraveled in recent years. On the other hand, long-term regulation of AVP neurons including that of the AVP gene expression appears to be mediated by the osmosensitive mechanisms within the vasopressinergic neurons themselves as well as the neural inputs from the aforementioned osmoreceptors. Our research group identified previously the transcription factors, CREB and AP1 (Fos/Jun), activated through the intracellular cAMP-dependent protein kinase A and calcium-dependent signaling, respectively, as positive regulators of the AVP gene expression. Unexpectedly, other CREB-family transcription factors related to the endoplasmic reticulum (ER) stress were recently shown to be involved in the regulation of the AVP gene, based on an emerging concept that osmotic stress triggers the ER stress. Regarding the AVP expression in the parvocellular neurons of the paraventricular nucleus, regulating the hypothalamo-pituitary-adrenal axis, another transcription factor, FosB, which has much longer intracellular half-life than c-Fos, may play an important role, especially during low glucocorticoid states.



Yasumasa Iwasaki, M.D., Ph.D.

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Yasumasa Iwasaki is a graduate of Tohoku University, Japan, in 1981. He worked as a post-doc fellow at University of Chicago (Prof. Gary Robertson) from 1988 through 1990, and then as a research associate at Harvard University (Prof. Joseph Majzoub) from 1990 through 1992. After coming back to Japan, he obtained his Ph.D. degree from Nagoya University in 1997, and was appointed an assistant professor and then lecturer at Nagoya University School of Medicine. In 2004, he moved to Kochi Medical School, Kochi University, and served as a lecturer. In 2009, he got promoted to Professor, Director of Health Care Center, Kochi University. He is specialized in the molecular biology of vasopressinergic neurons. He is an awardee of Kawakami Prize of Japan Neuroendocrine Society in 1999.

Molecular Regulation of CRH Gene Expression in Parvocellular Neurons

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Corticotropin releasing hormone (CRH) is essential for stress adaptation, mediating hypothalamic-pituitary-adrenal axis, behavioral and autonomic responses to stress. Activation of hypothalamic parvocellular CRH neurons depends on neural afferents from the brain stem and limbic system, leading to CRH release and synthesis. CRH gene transcription is required for restoration of mRNA levels following translation to protein. However, termination of this response is also essential for preventing pathology associated with chronic elevations of CRH and HPA axis activity. Transcriptional activation of the CRH gene depends on cyclic AMP/protein kinase A signaling and binding of phospho-CREB to a CRE at -270 in the CRH promoter. This CRE is essential for activation of the CRH promoter, and DNA methylation at its internal CpG reduces CREB binding to the promoter affecting CRH expression. Phospho-CREB alone cannot drive CRH transcription but it requires nuclear translocation and activation of the CREB co-activator, Transducer Of Regulated CREB activity (TORC) and its recruitment by the CRH promoter. Cyclic AMP activates TORC by inhibiting salt induced kinase (SIK) 2 allowing TORC dephosphorylation and nuclear translocation. The magnitude and duration of CRH neuron activation and transcriptional response is limited by glucocorticoids feedback inhibition, mainly through modulation of afferent pathways to the hypothalamic CRH neuron, as well as intracellular production of the repressor, Inducible Cyclic AMP Early Repressor (ICER). In addition, parallel to activating TORC, cyclic AMP induces SIK 1 expression, which may mediate nuclear TORC phosphorylation and inactivation, providing an additional mechanism for terminating CRH transcription.



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Greti Aguilera received her MD degree from the University of Chile in 1968. She was trained in Clinical Endocrinology, Department of Medicine, University of Chile, and in basic research on steroid biochemistry at the Department of Physiology and Biophysics. In 1975 she joined Dr. Kevin Catt's laboratory at NICHD as an International Fogarty Fellow, and in 1977 was appointed as a visiting scientist, and then as a senior researcher in 1984. From 1989 to 2014, she was chief of the Section of Endocrine Physiology, at the Developmental Endocrinology Branch in NICHD, leading a research program on the neuroendocrine mechanisms controlling homeostasis. Since 2014 she is scientist emerita at the same institution. Research contributions include identification of factors responsible for regulation of adrenal glomerulosa responsiveness to angiotensin II; characterization of the relative role of corticotropin-releasing hormone and vasopressin on the HPA axis adaptation during stress; characterization of the properties, signaling-transduction and regulation of the receptors for these peptide hormones, and the identification of novel mechanisms for the transcriptional regulation of CRH. Current work in Dr. Aguilera's laboratory focuses on the molecular mechanisms of positive and negative regulation of CRH transcription responsible for the rapid but transient responses to stress, the consequences of early life experiences and circadian and ultradian rhythms of glucocorticoid secretion on gene transcription, and the mechanisms determining pulsatile glucocorticoid secretion by the adrenal. She has about 250 publications in leading journals and books in endocrinology and neuroendocrinology.