Sleep, hypoxia, arousal and cardiovascular control: Mechanistic insights from rodents using optogenetics

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Obstructive sleep apnoea causes significant intermittent arterial hypoxemia and hypercapnia, and leads to cardiorespiratory stimulation and arousal from sleep. These adjustments in breathing, circulation and vigilance are initiated by feedback by O_2/CO_2 chemoreceptors such as the carotid body, and by airway mechano-, thermal and pressure sensors. Brainstem neuronal networks receive and integrate this respiratory afferent information, and activate physiological and behavioural effectors via a sequence of central neuronal connections that remain poorly defined. Identifying these key intermediate neuronal circuits, such as the circuitry responsible for the arousal by carotid body activation, is vitally important. So too is defining the endogenous factors and mechanisms that modulate the information flow along these circuits that shape neural gain (ie magnitude of autonomic response) and adaptive behaviour (ie arousal threshold). My talk will focus on recent efforts using optogenetic methodologies to test the contribution of specific brainstem pathways in rodents for the hypoxic or hypercapnic-induced cardiorespiratory stimulation and arousal from sleep. I will also present evidence that increased endogenous adenosine tone, via sleep deprivation or mild CNS hypoxia, suppresses brainstem circuits triggering arousal. This metabolic neuromodulator may underlie the increased arousal threshold seen in obstructive sleep apnoea.

C1 neurons mediate a stress-induced protection of renal ischemia/reperfusion injury

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The C1 neurons, a subset of catecholaminergic neurons located in the rostral ventrolateral medulla are activated by multiple stresses (pain, hypotension, hypoxia, hypoglycemia, and infection) and contribute to the autonomic and neuroendocrine responses elicited by these stimuli. We describe here a powerful anti-inflammatory effect of restraint stress, mediated by C1 neurons: protection against renal ischemia/reperfusion injury. Acute restraint stress for 10 min or optogenetic C1 neurons stimulation (5 Hz) for 10 min, which were applied 24 hours before induction of renal ischemia/reperfusion, protected mice from increase in plasma creatinine. The protection was reproduced by injecting splenic T cells that had been preincubated with noradrenaline or splenocytes harvested from stressed mice. Stress-induced protection of renal ischemia/reperfusion injury was absent in Chrna7 knockout ($a7nAChR^{-/-}$) mice and greatly reduced by destroying or transiently inhibiting C1 neurons. The protection conferred by stimulation of C1 neurons was eliminated by splenectomy, ganglionic-blocker administration or β2 adrenergic receptor blockade. Although stimulation of C1 neurons elevated plasma corticosterone and increased both vagal and sympathetic nerve activity, C1 neurons-mediated protection of renal ischemia/reperfusion injury persisted after subdiaphragmatic vagotomy or corticosterone receptor blockade. In conclusion, C1 neurons mediate the protective effect of stress against renal ischemia/reperfusion injury by activating the cholinergic anti-inflammatory pathway. In this particular case the cholinergic anti-inflammatory pathway seems to have been activated via a sympathetic rather than vagal route. This study also provides proof of principle that localized brain stimulation can produce beneficial anti-inflammatory effects.

Novel transgenic rat lines regulating the neuronal activity in rat vasopressin neuron by using Optogenetics and DREADDs technology

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Optogenetics or Chemogenetics (also known as DREADDs) give us new insight in the field of neuroscience. There are several ambitious studies using these techniques, however, to our knowledge, there is no successful study on arginine vasopressin (AVP) neurons.

We have previously generated a transgenic rat line which expresses the AVP-ChR2-enhanced green fluorescent protein (eGFP) fusion gene in the magnocellular neurosecretory cells (MNCs) of the hypothalamus. The eGFP fluorescence which indicates the expression of the ChR2 gene was observed in the supraoptic nucleus (SON) and the magnocellular division of the paraventricular nucleus (PVN). We succeeded in stimulating AVP neurons in the SON by blue light in this transgenic rat line, using whole-cell patch-clamp recordings.

Recently, we succeeded in generating a transgenic rat line which expresses hM3Dq tagged with mCherry fluorescence under the AVP promoter. The mCherry fluorescence which indicates the expression of the hM3Dq gene was observed in the SON and the magnocellular division of the PVN. The hM3Dq-mCherry was localized mainly in the membrane of the MNCs. The mCherry fluorescence were co-localized with AVP-like immunoreactive (LI) neurons, and were not co-localized with oxytocin-LI neurons. The number of Fos-LI neurons, which is the indicator of the neuronal activity, was robustly increased in the AVP-LI neurons in the SON and PVN 90min after intraperitoneal administration of clozapine-N-oxide (CNO), which activates hM3Dq receptors specifically. We would like to elucidate the role of endogenous AVP for diverse behavior, including drinking and feeding, using these transgenic rat lines.

Measurement of neuronal activity in conscious animal by *in vivo* photometry system

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In previous studies, orexin-deficient mice exposed to a stressor exhibited an attenuated fight-or-flight response, including increases in heart rate, respiration and blood pressure and stress induced analgesia. Although orexin neurons are essential factor of inducing autonomic response by stress, the orexin neuronal activity in presence of stressor and its physiological role remain unclear. In this study, we investigated whether orexin neuron activity would be changed in association with stress-induced autonomic responses. To record the real-time activity of orexin neurons from free-moving-mice, we established the fiber photometry system. We generated mice which selectively express G-CaMP6 in orexin neurons by injecting AAV-TetO(3G) G-CaMP6 into the orexin-tTA mice. Blue light emitted from the LED illuminates the G-CaMP6-expressing neurons in the brain. The green fluorescence from G-CaMP6 is then gathered by the same optic fiber. By using this fiber photometry system and telemeter systems, we succeeded in recording three parameters of temperature, heart rate and the orexin neural activity from the free-moving-mouse at the same time in real time. As expected, orexin neural activity immediately increased after several acute stress stimulation. Additionally, our data suggested strong correlation between orexin neural activity and heart rate. We propose that an increase in orexin neural activity should be a cause of autonomic changes associated with fight-or-flight response.