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The Research Grants Council of Hong Kong
NSFC/RGC Joint Research Scheme
Joint Completion Report

(Please attach a copy of the completion report submitted to the NSFC by the Mainland researcher)

Part A: The Project and Investigator(s)

1. Project Title

Orexin-induced modulation of activity-dependent synaptic plasticity is critical for the maturation of vestibular circuitry and functions

2. Investigator(s) and Academic Department/Units Involved

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	CHAN Ying-Shing (Professor)	WANG Jian-Jun (Professor)
Post	Professor	Professor
Unit / Department / Institution	School of Biomedical Sciences, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong	Department of Biological Science and Technology & State Laboratory of Pharmaceutical Biotechnology, School of Life Science, Nanjing
Contact Information	yschan@hku.hk	jjwang@nju.edu.cn
Co-investigator(s) *(with title and institution)*	SHUM Kwok-Yan Daisy (Professor) YUNG Wing-Ho (Professor) LAI Chun-Hong (Lecturer)	ZHUANG Qian-Xing (Lecturer) LI Bin (Lecturer)

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted*)
Project Start date	2015-01-01		
Project Completion date	2018-12-31		
Duration *(in month)*	48 months		
Deadline for Submission of Completion Report	2019-12-31		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. To determine the regulatory role of orexin on long-term synaptic plasticity in the VN and cerebellum.
2. To investigate if neonatal perturbation of orexin neurons in the hypothalamus or orexin synapses in the VN has any effect on (a) the tuning of synaptic efficacy in the VN and cerebellum, (b) developmental formation of spatial map, and (c) maturation of vestibular-related behaviors.
3. To examine if perturbation of orexin neurons in the hypothalamus or orexin synapses in the VN applied towards the end of the postnatal period has any effect on the mature animal.

5.2 Revised Objectives

Date of approval from the RGC: _____

Reasons for the change: _____

_____

6. Research Outcome

Major findings and research outcome

(maximum 1 page; please make reference to Part C where necessary)

Objective 1 – Regulatory role of neuromodulator

Hypothalamic orexinergic and histaminergic systems innerve the VN and cerebellum which is also known to send projection to the VN. But how these neuromodulatory systems affect VN neurons and subsequently participate in motor control are not well understood.

We found that histamine increased excitability and sensitivity of VN neurons through activation of histamine H2 receptors and their coupled HCN channels, contributing to histaminergic improvement of the vestibular-related motor behaviors. H1 receptors also promoted vestibular compensation following unilateral vestibular lesion. [*Frontiers in Cellular Neuroscience* 2017]

Progressive increase in postnatal expression of OX1R and OX2R was observed in the rat VN. Activation of these receptors by orexin or OX1/2R agonists suppressed the frequency of glutamate receptor-mediated miniature excitatory postsynaptic current (mEPSP) and $GABA_A$ receptor-mediated miniature inhibitory postsynaptic current (mIPSC). This shows the increasing potency of orexin in suppressing synaptic transmission in the maturing VN. [To be submitted to *J Neuroscience* in Jan 2020]

Objective 2 – Neonatal perturbation of orexinergic input to VN

(a) Effect on synaptic efficacy [To be submitted to *J Neuroscience* in Jan 2020]

Given the tight correlation between synaptic plasticity and structural modification of dendritic spines, we examined the maturation state of such spines in the VN. Orexin treatment at P1 significantly reduced the number of spines and the percentage of mature mushrooms but increased the proportion of immature filopodia. This suggests a delay in maturation of VN neurons. Since glutamergic synapses are found at head of spine, we also documented the expression of AMPA receptor subunits (GluA1 and GluA2) in the VN. Orexin or OX2R agonist treatment at P1 decreased these subunits while pre-treatment with OX1R antagonist had no effect. These suggest that activation of orexin receptors reduced excitability of VN neurons through attenuation of excitatory input by postsynaptic mechanism.

(b) Effect on formation of spatial map [To be submitted to *J Neuroscience* in Jan 2020]

To reveal the effect of suppressed vestibular development on ascending vestibular pathways for higher functions, the proportion of vestibular-related neurons in the anterodorsal nucleus (ADN) of the thalamus was examined. After robust vestibular stimulation by wobble rotation, the number of functionally activated ADN neurons was significantly attenuated in rats pre-treated at P1 with orexin or OX2R agonist. This deranged spatial map in the ADN indicated that the ascending vestibulo-thalamic pathway was not well formed with early over-activation of orexin receptors in the VN. On the other hand, OX1R antagonist treatment at P1 increased the number of vestibular-related neurons in the ADN.

(c) Effect on maturation of vestibular behaviours. [*Brain Structure Function; 2019;* To be submitted to *J Neuroscience* in Jan 2020]

Specific behavioral tests were conducted at different postnatal stages until adulthood. Neonatal treatment with OX2R agonist (1) postponed the emergence of innate reflexes (such as negative geotaxis, surface righting and air righting) during postnatal development; (2) impaired the performance of rota-rod test, balanced beam test, and spatial navigation at the adult stage. Similar postponement was observed following inhibition of GABAergic or glutamatergic transmission in the VN with bicuculline or MK801 respectively. On the other hand, neonatal treatment with OX1R antagonist accelerated postnatal emergence of innate reflexes and improved the learned motor coordination in the adult.

To investigate whether neonatal perturbation of orexin neurons in the lateral hypothalamus (sole source of orexin input to VN) has any effect on the maturation of vestibular-related behaviors, we stereotaxically injected an AAV-carrying shRNA construct against orexin into the lateral hypothalamus of P4 rats with an aim to inactivate the orexin neurons. At P13–21, these rats showed an accelerated acquisition of air-righting reflex when compared with the age-matched controls. At the adult stage, these rats also showed better spatial navigational performance than control animals. Taken together, we demonstrated that orexinergic modulation in the VN impacts developmental refinement of neural circuits that support vestibular-related behaviors.

Objective 3 – Postnatal time window of orexin action

To address whether orexinergic perturbation applied at late postnatal stage would also have long-lasting consequence, rats were exposed to orexin, OX2R agonist or OX1R antagonist at P14. It was found that P14 treatment did not result in any observable change in the battery of vestibular-related behavioral tests used. We therefore concluded that the critical period during which orexin can affect maturation of vestibular circuits ends before P14. [*To be submitted to J Neuroscience* in Jan 2020]

Potential for further development of the research and the proposed course of action *(maximum half a page)*

This project revealed that orexin modulates neonatal development of vestibular circuits. Activation of orexin receptors in VN suppressed both excitatory and inhibitory neurotransmission in VN. This delayed/ suppressed maturation of excitatory synapses in the VN, and deranged the pathways for dissemination of vestibular information to higher brain centers. We reason that such derangement caused the observed deficits in vestibular-related behavior. Real-time assessment of behaviour-correlated neuronal activity in higher brain centers by calcium imaging or multichannel recording will provide proof of circuit derangement and shed light on how vestibular input from the VN shapes behaviour.

Pilot experiments found bidirectional afferent innervation between the hypothalamus and cerebellum. To pinpoint the effect of orexin in cerebellar circuits, the drug delivery mode has to be modified to limit drug exposure to the cerebellum but not the VN. Our pilot results also indicated that blockade of orexin receptors affected the rewiring of adult vestibular circuits in response to lesion-induced loss of sensory input. These suggest that circuit plasticity in adults remains tunable beyond the developmental critical period, and opens new avenues for research into how such plasticity may be harnessed for rehabilitation after neurotrauma or curing of neurodevelopmental disorders.

Given the potent suppressive effect of orexin on both excitatory and inhibitory neurotransmission, experiments should be designed to investigate whether activation of orexin neurons is behavioral state specific. This would open new vistas in understanding the functional significance of neuromodulators.

7. The Layman's Summary

(describe <u>in layman's language</u> the nature, significance and value of the research project, in no more than 200 words)

This project reveals a novel neuromodulatory role of neuropeptide orexin in the development of circuits for processing spatial information. Activation of orexin receptors in brainstem neurons suppresses neural activity. This low activity state delays maturation of neurons within a critical period (two weeks after birth) in the neonatal rat. The altered maturation profile hindered consolidation of brain-wide pathways that support spatial cognition and caused deficits in spatial learning behaviour that persisted into adulthood.

We also offer a platform for therapeutic strategies that could rescue synaptic disorders in development and promote neurorehabilitation in adults.

Part C: Research Output

8. Peer-reviewed journal publication(s) arising directly from this research project

(Please attach a copy of each publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				Author(s) ***(bold** the authors belonging to the project teams and denote the corresponding author with an asterisk*)*	Title and Journal/ Book *(with the volume, pages and other necessary publishing details specified)*	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*						
2017				**Li B**, Zhang XY, Yang AH, Peng XC, Chen ZP, Zhou JY, **Chan YS**, **Wang JJ**, Zhu JN	Histamine increases neuronal excitability and sensitivity of the lateral vestibular nucleus and promotes motor behaviors via HCN channel coupled to H2 receptor. *Frontiers in Cellular Neuroscience* (2017) 10:300.doi: 10.3389/fncel.2016.00300.	December 31, 2016	No	Yes	Yes
2019				Ma CW, Kwan PY, Wu KL, **Shum DK*, Chan YS***	Regulatory roles of perineuronal nets and semaphorin 3A in the postnatal maturation of the central vestibular circuitry for graviceptive reflex. *Brain Structure and Function* (2019)224:613-626. doi: 10.1007/s00429-018-1795-x		Yes	Yes	Yes
			2019	Lam UT, Jiang Y, Kwan PY, Wu KL, Han L, Chua OW, **Shum DK, Wang JJ, Chan YS***	Orexin regulates developing rat vestibular circuitry for behaviours [*To be submitted to Journal of Neuroscience*]		Yes	Yes	No

9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered *(Please attach a copy of each delivered paper. All listed papers must acknowledge RGC's funding support by quoting the specific grant reference.)*

Month/Year/ Place	Title	Conference Name	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
March 2016/ Hong Kong	Developmental roles of orexin in the central vestibular system on acquisition of motor coordination and spatial recognition.	11th International Symposium on Healthy Aging: Science and Aging – An Era of Discovery: 60.	December 31, 2016	No	Yes	Yes
May 2016/ Hong Kong	Orexin modulates inhibitory synaptic transmission of vestibular nuclear neurons in rats.	Neuroscience Symposium 2016 & Scientific Conference of The Hong Kong Society of Neurosciences: 37.	December 31, 2016	No	Yes	Yes
November 2016/ San Diego	Modulatory role of orexin on synaptic transmission in the central vestibular system	46th Annual Meeting of the Society for Neuroscience (USA): 803.04	December 31, 2016	No	Yes	Yes
November 2016/ San Diego	Behavioral expression of orexin-modulated transmission in the vestibular nucleus of postnatal rats	46th Annual Meeting of the Society for Neuroscience (USA): 803.09	December 31, 2016	No	Yes	Yes
March 2017/ Hong Kong	Modulatory role of orexin on synaptic transmission in the central vestibular system	12th International Symposium on Healthy Aging: Science and Aging: 64		Yes	Yes	Yes

June 2017/ Hiroshima	Orexin modulates synaptic transmission in the central vestibular system	International Behavorial Neuroscience Society		Yes	Yes	Yes
July 2018/ Berlin	Orexin delays activity-dependent maturation of the vestibular system in rats	11th Federation of European Neuroscience Societies (FENS) Forum of Neuroscience		Yes	Yes	Yes
March 2019/ Kobe	Postnatal refinement of circuit plasticity for spatial navigation **[Invited talk]**	9th Congress of Federation of Asian-Oceanian Physiological Sciences (FAOPS)		Yes	Yes	Yes

10. Student(s) trained *(Please attach a copy of the title page of the thesis.)*

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Ulysses Tsz-Fung LAM	MPhil	January 2015	May 2017
Ivy Yuan JIANG	MPhil	September 2015	January 2018

11. Other impact *(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)*

N.A.