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

Novel Functions of Spexin as a Regulator for Reproduction and Feeding in Fish Model.

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

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Wong Anderson On-Lam	Lin Haoran
Post	Professor	Professor
Unit / Department / Institution	School of Biological Sciences The University of Hong Kong	School of Life Science Sun Yatsen University
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Co-investigator(s) *(with title and institution)*	NA	Zhang Yon (Asso. Prof., School of Life Science, Sun Yatsen University)

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	01-Jan-2013	NA	NA
Project Completion date	31-Dec-2016	NA	NA
Duration *(in month)*	48 months	NA	NA
Deadline for Submission of Completion Report	30-Dec-2017	NA	NA

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

(1) Using goldfish as a model for modern-day bony fish to confirm the central expression of the newly identified neuropeptide SPX and establish its distribution profile in the hypothalamo- pituitary axis & related brain areas with possible association with neurons expressing GnRH & feeding regulators in the brain and gonadotrophs in the pituitary.

(2) Using in vivo & in vitro approaches to study the functional role of SPX in LH regulation & reproductive performance in goldfish through direct actions in the pituitary and/or indirect actions through modulation of GnRH expression in the brain or functional interactions with sex steroids from the gonad.

(3) To provide in vivo & in vitro evidence that SPX expression in goldfish can be induced by feeding, which in turn serves as a satiety signal to suppress feeding behavior & food intake by direct actions to inhibit basal feeding/block the orexigenic effects of feeding stimulators and/or indirect actions to modify gene expression of various feeding regulators in brain areas involved in feeding control.

5.2 Revised Objectives

Date of approval from the RGC: _____

Reasons for the change: _____

_____

Not applicable.

6. Research Outcome

Major findings and research outcome
(maximum 1 page; please make reference to Part C where necessary)

(I) Structural characterization & tissue expression of SPX: The full gene of goldfish SPX was cloned & confirmed to be composed of 4 introns and 5 exons. The 14 a.a. mature peptide encoded was found to be highly conserved from fish to mammals with only one a.a. substitution. As revealed by CD & NMR spectroscopies, the solution structure of SPX mature peptide was confirmed to be in the form of a C-terminal helical peptide with Lys^{11} as the only charged residue on the molecular surface. Using RT-PCR & real-time PCR, different isoforms of SPX transcripts were detected and shown to be ubiquitously expressed at tissue level, including different brain areas & pituitary. Using LC/MS/MS, SPX mature peptide was identified in the brain-pituitary axis of goldfish & this finding was further substantiated by the detection of SPX immunoreactivity (IR) in neurons within brain areas with expression of GnRH & various feeding regulators, including the telencephalon, hypothalamus and optic tectum. In goldfish pituitary, SPX IR signals were also found in nerve fibres in close proximity to gonadotrophs located in the pars distalis. These findings have provided new information for SPX in terms of its genomic organization, molecular structure and tissue expression related to its potential functions in the brain & pituitary in fish model.

(II) Functional role of SPX in reproduction: In goldfish, a gradual drop in SPX expression in the hypothalamus was noted when the fish became sexually mature during seasonal reproductive cycle and similar findings were also observed in zebrafish. In sexually mature goldfish, SPX treatment by IP injection or static incubation of pituitary cells was found to reduce LH release without altering LHβ & GtHα gene expression at pituitary level. In female fish at the same stage, SPX expression in the hypothalamus could be elevated by castration and the effect was blocked by estrogen replacement. In similar studies with fish in sexual regression & recrudescence, interestingly, SPX was shown to induce LH release & the effect was mediated by pituitary GalR2b coupled to PLC/PKC, Ca^{2+}/CaMK-II, MAPK and PI3K/Akt but not cAMP/PKA cascades. In these experiments, the LH-releasing effect of SPX was not additive to GnRH but could be inhibited by dopamine D2 activation at pituitary level or co-treatment with testeosterone/estrogen. Given that ICV injection of SPX was not effective in altering plasma LH level/affecting GnRH expression in the hypothalamus, LH regulation by SPX probably is mediated by direct action acting on the goldfish pituitary. Our findings, as a whole, provide evidence that SPX can play a role in goldfish reproduction by modulating LH secretion presumably via seasonal interactions with gonadal steroids & other LH regulators. Of note, in parallel studies with grouper & zebrafish, the reproductive function/ LH regulation by SPX was not apparent, suggesting that the functional role of SPX in reproductive control in fish models may be species-specific.

(III) Functional role of SPX in feeding control: In goldfish, SPX expression in the telencephalon, hypothalamus & optic tectum could be up-regulated by feeding. Meanwhile, SPX treatment by IP & ICV injection were shown to inhibit feeding behaviour & food consumption with parallel rises in anorexigenic factors (including POMC, CART, CCK, MCH & CRH) & down-regulation of orexigenic signals (including NPY, AgRP, apelin & orexin) in the brain areas with SPX expression after feeding. In parallel studies, food intake induced by NPY & orexin was blocked by SPX co-treatment in vivo and the differential effects on gene expression of various orexigenic & anorexigenic factors after SPX treatment were also observed in goldfish brain cell culture prepared from the same brain areas. The postprandial rise in SPX expression at hypothalamic level & SPX modulation of feeding & central expression of feeding regulators had also been confirmed in grouper & zebrafish. Using both in vivo & in vitro studies, the insulin signal caused by glucose rise in circulation after food intake was confirmed to be the functional link between feeding & SPX expression. In goldfish, insulin by acting as an endocrine signal could induce SPX expression in telencephalon, hypothalamus & optic tectum & similar effects were mimicked by glucose treatment. Interestingly, insulin was also found to be expressed at high level in goldfish liver under the influence of blood glucose, which could act as an autocrine/paracrine signal to increase SPX expression & secretion at the hepatic level. The SPX responses in the brain & liver were mediated mainly by insulin receptor & to a lesser extent by IGF-I receptor functionally coupled to P_{38} ^{MAPK} & PI3K/Akt pathways. The subsequent rise of SPX in circulation together with the central expression of SPX under insulin induction may act together to trigger the differential regulation of feeding signals (both orexigenic & anorexigenic) in brain areas involved in appetite control in goldfish. These findings, taken together, provide evidence that SPX can serve as a novel satiety factor in fish model by functional coupling with insulin & glucose signals triggered by food intake.

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

(I) Further development of SPX study in zebrafish: Since the use of mini-osmotic pump for long-term study of SPX treatment in goldfish was proven to be not appropriate (as the size of the osmotic pump is still too big), our research partners in China, Profs Hoaran Lin & Yong Zhang, have extended our SPX study to zebrafish. Recently, they have been successful in establishing the zebrafish mutant with SPX knockout. Based on their initial findings, SPX knockout did not affect puberty onset & gonadal functions in zebrafish. However, the mutant did exhibit increased appetite with parallel rise in AgRP expression in the hypothalamus but with no effect on body growth. Nevertheless, detectable rises in serum protein, triacylglycerol & cholesterol were noted, suggesting that the knockout model can be used for future study of SPX research in fish model.

(II) Extension of SPX research to mouse model: Based on our studies in goldfish, the functional role of SPX in feeding control was also extended to the mouse model in our laboratory. With the support of a HMRF grant, studies have been initiated in the mouse model to (i) set up the NMR solution structure & its docking model with GalR2 & GalR3 receptors of mouse origin, and (ii) work out the mechanisms for SPX regulation of appetite control in mammalian model. Based on our initial studies, the solution structure of mouse SPX is pretty much identical to that of goldfish SPX and the helical peptide of SPX could insert in a "vertical manner" into the binding pocket formed by clustering of the 7 transmembrane domains of GalR2 & GalR3. Similar to goldfish, SPX expression & secretion could be induced by feeding in mouse entrained to a one-meal-per-day feeding schedule. However, the site of SPX expression was found in the stomach but not in the hypothalamus, liver or omental fat. Besides, ICV injection of SPX was also effective in reducing food intake with parallel inhibition of NPY, AgRP & ghrelin receptor expression in the hypothalamus. We are still in the process of working out the receptor specificity (GalR2/3) for feeding control by SPX & functional role of insulin signal in SPX regulation in the mouse model.

7. The Layman's Summary

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

Spexin (SPX), a peptide first identified by bioinformics, has emerged as a neuropeptide with pleiotropic functions but little/no information is available regarding its physiological role in lower vertebrates. Using goldfish as a model, the genomic organization, NMR solution structure and tissue expression of SPX have been characterized in fish species. Within the CNS, SPX expression could be detected in brain areas with expression of GnRH & feeding regulators as well as in nerve fibres in close proximity to gonadotrophs in the anterior pituitary. Consistent with these findings, our in vivo and in vitro studies have confirmed that SPX can play a functional role in reproduction and feeding control in goldfish. In this case, SPX produced in the hypothalamus probably could exert a direct effect at pituitary level to regulate LH release according to different stages of the reproductive cycle via seasonal interactions with gonadal steroids and other LH regulators expressed in the brain-pituitary axis. Meanwhile, SPX could also serve as a novel satiety factor in goldfish. Its expression in brain areas involved in appetite control could be induced by food intake to differentially modulate the central expression of orexigenic & anorexigenic signals to trigger subsequent inhibition on feeding behaviour.

(Word count: 199)

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)*						
2013				M.K.H. Wong, K.H. Sze, T. Chen, C.K. Cho, Henry C.H. Law, I.K. Chu & A.O.L. Wong *	Goldfish Spexin: Solution structure and novel function as a satiety factor in feeding control. Am. J. Physiol. Endo & Metab 305:E348 - E366	2015	Yes	Yes	Yes
2013				Y. Liu, S. Li, X. Qi, W. Zhou, X. Liu, H. Lin, Y. Zhang * & C. H.K. Cheng *	A novel neuropeptide in suppressing luteinizing hormone release in goldfish, Carassius auratus. Mol Cell Endocrinol 374: 65-72.	2015	Yes	Yes	Yes

2013				C. Sun, M. He, W.K.W. Ko, & A.O.L. Wong *	Gene expression of luteinizing hormone receptor in carp somatotrophs differentially regulated by local actions of gonadotropin and dopamine D1 receptor activation. Mol Cell Endocrinol 374:22-34.	2015	Yes	Yes	Yes
2013				Q. Jiang & A. O.L. Wong *	Signal transduction mechanisms for autocrine/paracrine regulation of somatolactin α secretion and synthesis in carp pituitary cells by somatolactin α and β. Am. J. Physiol. Endo & Metab 304:E176 - E186.	2015	Yes	Yes	Yes

2014				Q. Jiang, M. He, W.K.O. Ko & A.O.L. Wong *	Kisspeptin induction of somatolactin α release in goldfish pituitary cells: Functional role of cAMP/PKA-, PLC/PKC-, and Ca^{2+}/calmodulin-dependent cascades. Am J Physiol Endo & Metab 307:E872-884.	2015	Yes	Yes	Yes
2014				G. Hu, C. Lin, M. He & A.O. L. Wong *	Neurokinin B and reproductive functions: -"KNDY neuron" model in mammals and the emerging story in fish. Gen Comp Endocrinol 208:94-108.	2015	Yes	Yes	Yes

2014				C. Sun, M. He, W.K.W. Ko, & A.O.L. Wong *	Mechanisms for luteinizing hormone induction of growth hormone gene transcription in fish model: Crosstalk of the cAMP/PKA pathway with MAPK- and PI3K-dependent cascades. Mol. Cell Endocrinol 382:835-850.	2015	Yes	Yes	Yes
2014				G. Hu, M. He, W.K.W. Ko, C. Lin, & A.O. L. Wong *	Novel pituitary actions of TAC3 gene products in fish model: - Receptor specificity and signal transduction for prolactin and somatolactin α regulation by neurokinin B (NKB) and NKB-related peptide in carp pituitary cells. Endocrinology 155:3582-3596.	2015	Yes	Yes	Yes

2015				C. Lin, X. Jiang, G. Fu, W.K.W. Ko, & A.O.L. Wong *	Grass carp prolactin: Molecular cloning, tissue expression, intrapituitary autoregulation by prolactin and paracrine regulation by growth hormone and luteinizing hormone. Mol Cell Endocrinol 399:367-283	NA	Yes	Yes	Yes
2016				X. Jiang, J. Xiao, M. He, A. Ma, & A.O.L. Wong *	Type II SOCS as feedback repressor for GH-induced IGF1 expression in carp hepatocytes. J Endocrinol 229:171-186	NA	Yes	Yes	Yes
2016				G. Hu, M. He & A.O.L. Wong *	Novel functional role of NK3R expression in the potentiating effects on somatolactin α autoregulation in grass carp pituitary cells. Sci Rep 6:36102-36114 / doi: 10.1038	NA	Yes	Yes	Yes

2016				S. Li, Q. Liu, L. Xiao, H. Chen, G. Li, Y. Zhang, & H. Lin *	Molecular cloning and functional characterization of spexin in orange-spotted grouper (Epinephelus coioides). Comp Biochem Physiol B 196-197:85-91	NA	Yes	Yes	Yes
2017				A. Ma, M. He, J. Bai, M.K.H. Wong, W.K.W. Ko, & A.O.L. Wong *	Dual role of insulin in spexin regulation : Functional link betwee food intake and spexin expression i fish model. Endocrinolc gy 158:560-57	NA	Yes	Yes	Yes
2017				G. Hu, M. He, W.K.W. Ko, & A.O.L. Wong *	TAC1 gene products regulate pituitary hormone secretion and gene expression in prepubertal grass carp pituitary cells. Endocrinology 158:1-22.	NA	Yes	Yes	Yes

2017				B. Zheng, S. Li, Y. Liu, Y. Li, H. Chen, H. Tang, X. Liu, H. Lin, Y. Zhang, & C. H. K. Cheng *	Spexin suppress food intake in zebrafish: Evidence from gene knockout study. Sci Rep 7:14643 / doi: 10.1038	NA	Yes	Yes	No
		Under review		A. Ma, J. Bai, M. He, & A. O. L. Wong *	) Spexin as a neuroendocrine signal with emerging functions in mammals and fish models. Gen Comp Endocrinol (submitted & currently under review).	NA	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)*
Mar/2012/ Kauala Lumpur, Malaysia	Spexin as a novel satiety factor in fish model. (State-of-the- Art Lecture)	The 7th Congress of Asian & Oceania Society of Comparative Endocrinology	2015	Yes	Yes	No
Sept/2012/ Buenos Aires, Argentina	Novel function of spexin as a feeding inhibitor in fish model: del: A pilot study in goldfish.	International Symposium on Fish Endocrinology	2015	Yes	Yes	No

July/2013/ Barcelona, spain	Novel mechanisms for signal termination of growth hormone (GH) receptor: - PIAS1 as a feedback repressor for GH-induced IGF-I gene transcription via JAK2/STAT5 signaling. (State-of-the-Art Lecture)	The 17th International Congress of Comparative Endocrinology	2015	Yes	Yes	No
Mar/2014/ Keelung, Taiwan	Novel pituitary actions of TAC3 gene products in fish model.	The 7th Intercongress Symposium of Asian & Oceania Society of Comparative Endocrinology	2015	Yes	Yes	No
April/2016/ Boston, MA, USA	Insulin as a postprandial signal for spexin induction in fish model: - Signal transduction and evidence of a peripheral spexin component.	The 98th Annual Meeting of the Endocrine Society	NA	Yes	Yes	No
June/2016/ Seoul, Korea	Synergism of IGF and TAC3 gene products in somatolactin α regulation: Pituitary type III neurokinin receptor expression and intrapituitary feedback by somatolactin α. (State-of-the-art lecture)	The 8th Congress of the Asian & Oceania Society for Comparative Endocrinology	NA	Yes	Yes	No
April/2017/ Orlando, Florida, USA	Evidence for spexin as a novel luteinizing hormone (LH)-releasing factor in goldfish via direct action at pituitary level: - Receptor specificity, signal transduction and interactions with LH regulators in fish model.	The 99th Annual Meeting of the Endocrine Society	NA	Yes	Yes	No

June/2017/ Alberta, Canada	Insulin as a functional link between food intake and spexin expression: Recent progress on spexin as a satety factor in fish model. [Invited symposium presentation]	The 18th International Congress of Comparative Endocrinology	NA	Yes	Yes	No

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
Jiang Xue	PhD	Sept 2009	Sept 2013
Wong Wei, Wade	M.Phil	Sept 2011	Aug 2013
Wong Ka Hei, Matthew	M.Phil	Sept 2011	Aug 2013
Hu Guangfu	PhD	Sept 2010	Nov 2014
Chen Shuang	PhD	Sept 2010	Feb 2015
Ma Ani	PhD	Sept 2013	Nov 2017

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

Not Applicable.