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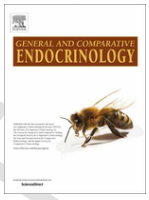

Minireview

Spexin as a neuroendocrine signal with emerging functions

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ABSTRACT

Spexin (SPX), a novel peptide coevolved with the galanin/kisspeptin family, was first identified by bioinformatics prior to its protein purification/functional studies. Its mature peptide is highly conserved among different vertebrate classes. Based on the studies in mammals and fish models, SPX was found to be widely distributed at tissue level, secreted into systemic circulation, identified at notable levels in central nervous system and peripheral tissues, and has been confirmed/implicated in multiple functions in different tissues/organs, suggesting that SPX may serve as a neuroendocrine signal with pleotropic functions. In this article, different isoforms of SPX and their binding with their cognate receptors GalR2 and GalR3, the biological functions of SPX reported in mammals including GI tract movement, energy balance and weight loss, fatty acid uptake, glucose homeostasis, nociception and cardiovascular/renal functions, as well as the recent findings in fish models regarding the role of SPX in reproduction and feeding control will be reviewed with interesting questions for future investigations.

1. . Introduction

Spexin (SPX), also referred to as neuropeptide Q, is a polypeptide encoded by the C12orf29 gene located in chromosome 12 of the human genome (Wan et al., 2010). It was first identified in 2007 by data mining of human proteome with hidden Markov method (Mirabeau et al., 2007) and the finding was later confirmed by another group using evolutionary probabilistic models based on the genome database reported in different vertebrates (Sonmez et al., 2009). It is one of the recent examples for identification of novel peptides by bioinformatics prior to their protein purification/functional studies. Using sequence analysis and comparative synteny, SPX has been recently proposed to be coevolved with galanin (Gal) and kisspeptin (Kiss) and classified as a member of the Gal/Kiss peptide family (Kim et al., 2014). Based on the studies in fish (e.g., goldfish) and mammals (e.g., rodents & human), SPX are known to be widely expressed in different tissues/organs, including the brain, heart, lung, liver, thyroid, adrenal, muscle, body fat, ovary, testis, pancreas, stomach and different parts of the GI tract (Mirabeau et al., 2007; Porzionato et al., 2010; Wong et al., 2013). In representative species, e.g., human (Walewski et al., 2014) and goldfish (Ma et al., 2017), SPX immunoreactivity can be detected in systemic circulation and modified by physiological status (e.g., obesity) or hormonal signals (e.g., insulin). Meanwhile, notable levels of SPX, both transcript and protein signals, can also be located in different brain areas as well as in neurons/cell bodies within the hypothalamus and other brain nuclei, e.g., in the rat (Porzionato et al., 2010) and goldfish (Liu et al., 2013). Consistent with its wide range of tissue distribution, increasing functions of SPX start to emerge in recent years together with the new information for SPX isoforms and their receptors, suggesting that the newly discovered peptide may serve as a neuroendocrine signal with pleotropic functions. In this article, the structural aspects of SPX and its binding to different SPX receptors, the biological functions of SPX reported in mammals related to smooth muscle contraction in GI tract, energy balance and weight loss, fatty acid uptake, glucose homeostasis, nociception and cardiovascular/renal functions, as well as the recent findings in fish models regarding the role of SPX in reproduction and appetite control will be reviewed with stress on the unexplored areas still await for future investigations.

2. . Spexin and its binding with GalR2 and GalR3

The structural organization of the coding sequence of SPX is well conserved from fish to mammals with a hydrophobic signal peptide followed by a linker region and the 14 a.a. mature peptide of SPX flanked by RR/KR and GRR dibasic cleavage sites (Liu et al., 2013; Mirabeau et al., 2007; Sonmez et al., 2009; Wong et al., 2013) (Fig. 1A). Based on the expression study of SPX in pancreatic βTC3 cells, protein signals of SPX could be located in the secretory vesicles with insulin as well as in the culture medium, suggesting that SPX can be

Abbreviations: SPX, spexin; Gal, galanin; Kiss, kisspeptin; LH, luteinizing hormone; GalR1, type I galanin receptor; GalR2, type II galanin receptor; GalR3, type III galanin receptor; hs-CRP, high-sensitivity C-reactive protein test index; HbAlc, haemoglobin A1C

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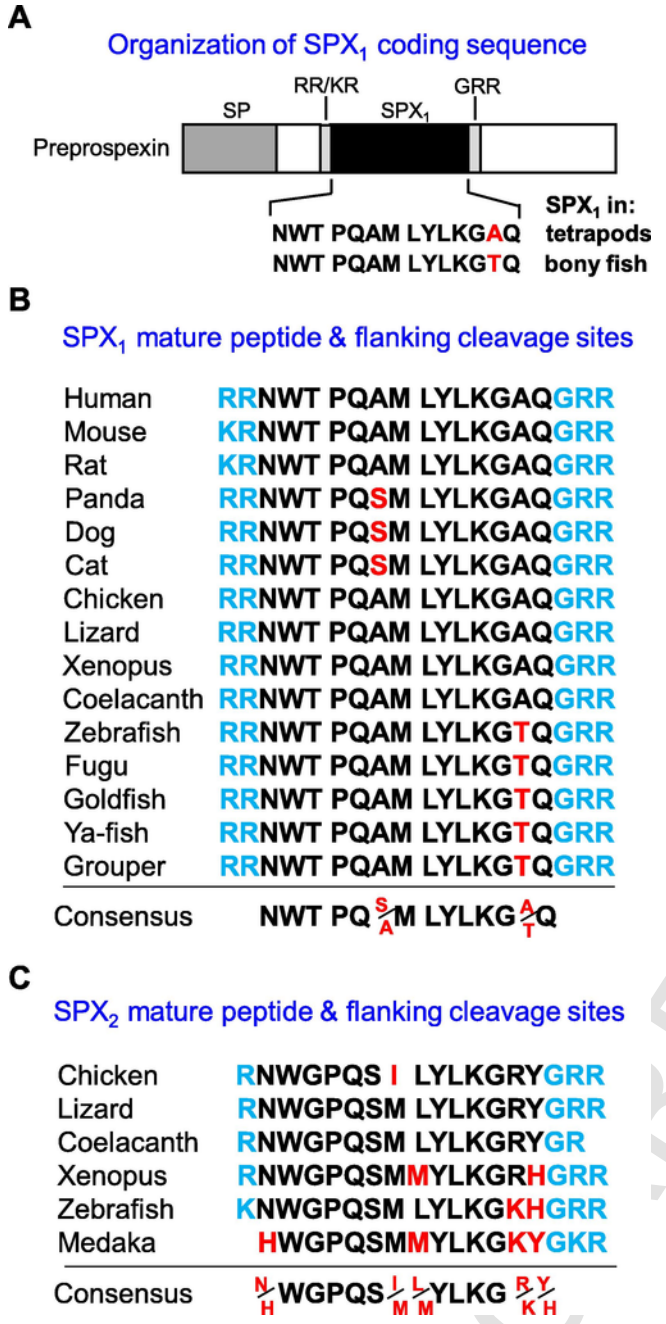

Fig. 1. Protein sequences of SPX reported in vertebrate species. (A) Structural organization of the protein coding sequence of SPX_1 reported in tetrapods and fish models. The coding sequence of SPX_1 is composed of a signal peptide (SP) followed by a linker region, SPX_1 mature peptide flanked by dibasic protein cleavage sites (RR/KR & GRR) and a C-terminal tail region. The consensus sequence of tetrapod and fish SPX_1 mature peptide has only one a.a. substitution at position 13. The structural organization of the protein coding sequence of SPX_2 (e.g., in zebrafish) is highly comparable with that of SPX_1. Alignment of (B) SPX_1 and (C) SPX_2 mature peptide and its flanking monobasic/dibasic cleavage sites reported in different species. The sequence alignment was conducted using Clustal-W algorithm with residues labelled in red for a.a. substitution(s) found in the consensus sequence of SPX_1/SPX_2 mature peptide and in blue for the flanking monobasic/dibasic cleavage sites. (Figures modified from Wong et al., 2013; Kim et al., 2014.) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

processed properly and released by exocytosis at the cellular level (Mirabeau et al., 2007). In mammals, except for panda, dog and cat with a Ala^6 to Ser^6 substitution, the a.a. sequence of SPX mature peptide is identical among different species with the consensus sequence of NWTPQAMLYLKGAQ (Fig. 1B). The same sequence can also be found in representative species of bird, reptile and amphibian as well as in coelacanth, suggesting that the mature peptide of SPX is well preserved during the evolution of tetrapod lineage. In ray-finned fishes, a Ala^{13} to Thr^{13} substitution is observed in SPX mature peptide and the consensus sequence NWTP QAMLYLKGTQ can be found in all the species examined to date (referred to as "fish SPX"). As revealed by NMR spectroscopies, the solution structure of fish SPX is composed of an N-terminal random coil from Asn^1 to Pro^4 followed by an extended α helix from Gln^5 to Gln^{14} in the C-terminal and the molecular surface of fish SPX is largely hydrophobic with Lys^{11} as the only charged residue, which is believed to play a key role in receptor binding/activation. At present, the solution structure of mammalian SPX is not available but knowledge-based modelling has predicted that the mammalian SPX with a Thr^{13} to Ala^{13} substitution also exists in the form of a helical peptide highly comparable if not identical to the 3D structure of fish SPX (Lin et al., 2015).

Based on recent data mining and comparative synteny, a second form of SPX, namely SPX_2, has been identified in species ranging from fish to bird but not in mammals (the first form of SPX reported previously is now renamed as "SPX_1") (Kim et al., 2014). In respective species, SPX_2 is encoded by a separate gene and the structural organization of its coding sequence is comparable to SPX_1, except that its mature peptide is flanked by R and GRR/GKR cutting sites, and depending on the species, up to 4 a.a. substitutions could be found among position 1, 3, 6, 7, 13 and 14 compared to the corresponding sequence of SPX_1 (Fig. 1C). Despite the sequence variations found in the signal peptide, linker region and C-terminal tail, the mature peptides of the two SPX are highly conserved, suggesting that SPX has been evolved under strong selection pressure and may be involved in important functions essential for survival. Since GRR/GKR is the target site for peptidylglycine α-amidating monooxygenase (Merkler, 1994) and the motif is well-conserved in the two forms of SPX, the C-terminal of SPX mature peptide is believed to be α-amidated and the modification may have an effect on its peptide stability/receptor binding.

Recently, type II (GalR2) and type III Gal receptors (GalR3) have been confirmed to be the cognate receptors for SPX (Kim et al., 2014), which is consistent with the idea that SPX is a member of the Gal/Kiss peptide family. Apparently, SPX_1 from mammals and SPX_1 and SPX_2 from *Xenopus* and zebrafish could not exhibit binding affinity nor the ability to activate type I Gal receptor (GalR1). In the same study, human GalR2 was shown to be promiscuous for $SPX_{1/2}$ and Gal binding/activation with a higher affinity for Gal but human GalR3 was found to be more selective for SPX_1 and SPX_2 and the preference for SPX over Gal was also observed in GalR3 from *Xenopus* (Kim et al., 2014). Of note, two forms of GalR2, GalR2a and GalR2b, could be identified in lower vertebrates including fish and amphibians (Fig. 2), which might be the result of whole genome duplication occurred during the early phase of vertebrate evolution (Yant and Bomblies, 2015). However, the corresponding sequence for GalR3 was not found in the genome databases of fish models. As revealed by functional expression in cell line (e.g., HEK293 cells), GalR2a from *Xenopus* and zebrafish could still retain the promiscuity/preference for Gal binding but the corresponding receptors for GalR2b were found to be highly selective for SPX_1 and SPX_2, despite a cross-activity with Gal at high doses (Kim et al., 2014). These findings, as a whole, raise the possibility that the GalR2b in lower vertebrates may be the functional equivalence of GalR3, which was evolved prior to the appearance of GalR3 in mammalian lineage. Although GalR2a and GalR2b can also be found in avian species (e.g.,

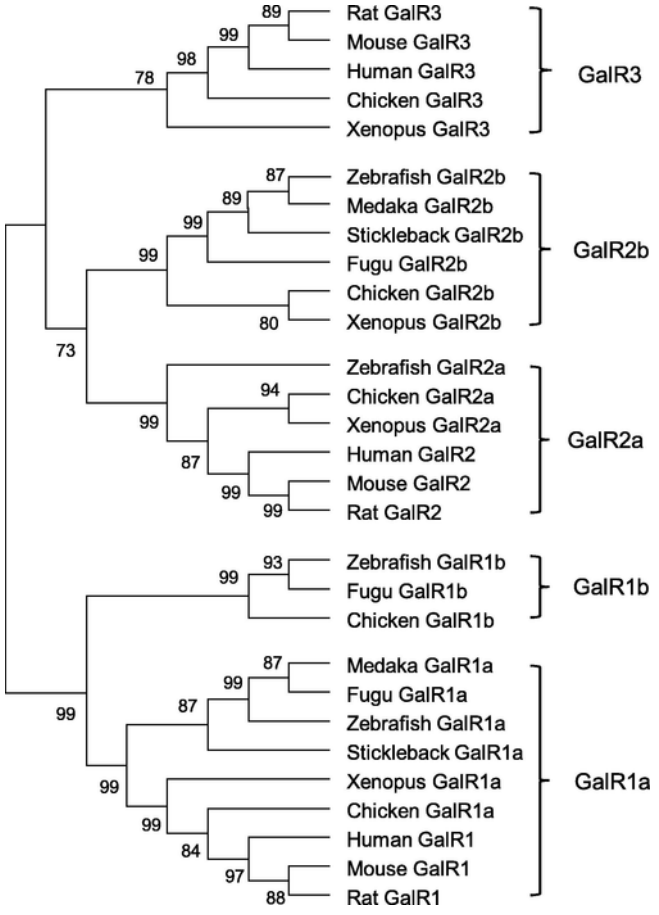

Fig. 2. Phylogenetic analysis of different isoforms of galanin receptors (GalR) identified in different vertebrate species. The open reading frame of individual isoforms of GalR, including GalR1, GalR2 and GalR3, was subjected to phylogenetic analysis using neighbour-joining method with MEGA 5.1. The numbers presented at the branch points of the dentrogram are percentage scores based on 1000 bootstraps. The nucleotide sequences of GalR1, GalR2 and GalR3 reported in different species were downloaded from the GenBank.

chicken), their binding properties for $SPX_{1/2}$ and Gal have not been characterized.

3. . Emerging functions of spexin in mammals and fish models

Given that (i) SPX_2 has not been identified in mammals and (ii) other than the limited studies on GalR2/3 receptor binding/activation, no information is available regarding the physiological role or biological actions of SPX_2, our subsequent discussion on the emerging functions of SPX will only focus on SPX_1 reported in mammals and fish models (Fig. 3).

4. . Biological functions of spexin in mammals

Regarding the emerging functions of SPX, its effects on energy balance and glucose homeostasis for sure are the exciting development in the field. In 2010, a microarray study with the objective to identify the "dysregulated genes" in adipose tissue in obese subjects revealed that C12orf29 coding for SPX was the most down-regulated gene (>14-fold decrease) in both omental and subcutaneous fat (Walewski et al., 2010). In subsequent studies, SPX expression in adipose tissue as well as circulating level of SPX was found to be much reduced in individual with obesity (Kumar et al., 2016; Walewski et al., 2014). Furthermore, based on the serum data obtained from obese versus non-obese subjects, a negative correlation was observed between circulating levels of SPX and leptin (Kolodziejskii et al., 2017) and a low level of serum SPX has been proposed to be a biomarker for both childhood (Kumar et al., 2016) and adult obesity (Walewski et al., 2014). Recently, a similar study with circulating level of C-reactive protein also reveals a positive correlation of "low SPX with high leptin" in serum with a higher hs-CRP index in obese adolescents, suggesting that the SPX signal may be linked with the risk factor for cardiovascular disease (Kumar et al., 2017). In mouse model with dietary-induced obesity, SPX treatment can consistently reduce body weight (Ge et al., 2016; Walewski et al., 2014), probably by inhibiting caloric intake together with increasing locomotion and lipid oxidation during the dark phase (Walewski et al., 2014). In the same animal model, in vivo treatment or in vitro incubation of isolated adipocytes with SPX can also inhibit fatty acid uptake into white adipose tissue (Walewski et al., 2014) and similar phenomenon has been recently reported in the liver, which can lead to a drop in hepatic lipid content by reducing fatty acid uptake into hepatocytes (Ge et al., 2016). These findings, as a whole, indicate that SPX may serve as an adipokine with function on energy balance/metabolism and with potential application for obesity treatment (e.g., for weight control & fatty liver).

Given that obesity is associated with a strong prevalence of diabetes (Agha and Agha, 2017), which is known to have a strong impact on public health, the role of SPX in glucose homeostasis is also a major subject for perusal regarding the novel function of SPX. In human, pancreatic islets are known to have SPX expression, both at the transcript and protein level (Gu et al., 2015). In pancreatic islet cells (e.g., βTC3 cell line), SPX immunoreactivity has been shown to be co-localized with insulin in secretory vesicles (Mirabeau et al., 2007), implying that SPX can be released together with insulin. In patients with type 2 diabetes, serum level of SPX has been reported to be attenuated (by ~44%) with a negative correlation to the circulating levels of blood glucose, haemoglobin A1C (HbAlc), triglyceride and LDL/cholesterol, suggesting that SPX may play a role in glucose and lipid metabolism (Gu et al., 2015). A recent study with obese female also reveals that serum SPX can exhibit a similar negative correlation with circulating levels of insulin and glucagon, which raises the possibility that SPX may be involved in glucose regulation and insulin resistance (Kolodziejskii et al., 2017). This idea is also consistent with the findings in obese mouse with type 2 diabetes, in which SPX treatment not only can reduce body weight but also improve glucose tolerance with reduction of insulin resistance and HbAlc level (Ge et al., 2016). However, a similar correlation of serum SPX with diabetes or with circulating parameters for insulin sensitivity/blood lipids cannot be demonstrated in young adolescent with type 2 diabetes (Hodges et al., 2017) and further investigations are clearly warranted to establish the role of SPX as a metabolic regulator or a biomarker for glucose control in mammals.

In rodents (e.g., rat), SPX signals, both at transcript and protein levels, have been demonstrated in the stomach as well as in different regions of the intestine (Mirabeau et al., 2007; Porzionato et al., 2010). In rat stomach, SPX is expressed in the submucosal layer with smooth muscle and stomach contraction can be induced by SPX in vitro in a stomach explant culture (Mirabeau et al., 2007). A recent study in the mouse also reveals that IP injection of SPX is effective in stimulating gut motility in small intestine and colon and these stimulatory effects are mediated via GalR2 activation of L-type Ca^{2+} channels expressed in the smooth muscle layer (Lin et al., 2015). In the same study, emptying the gut content by food deprivation can also reduce SPX gene expression in small intestine and colon. In human,

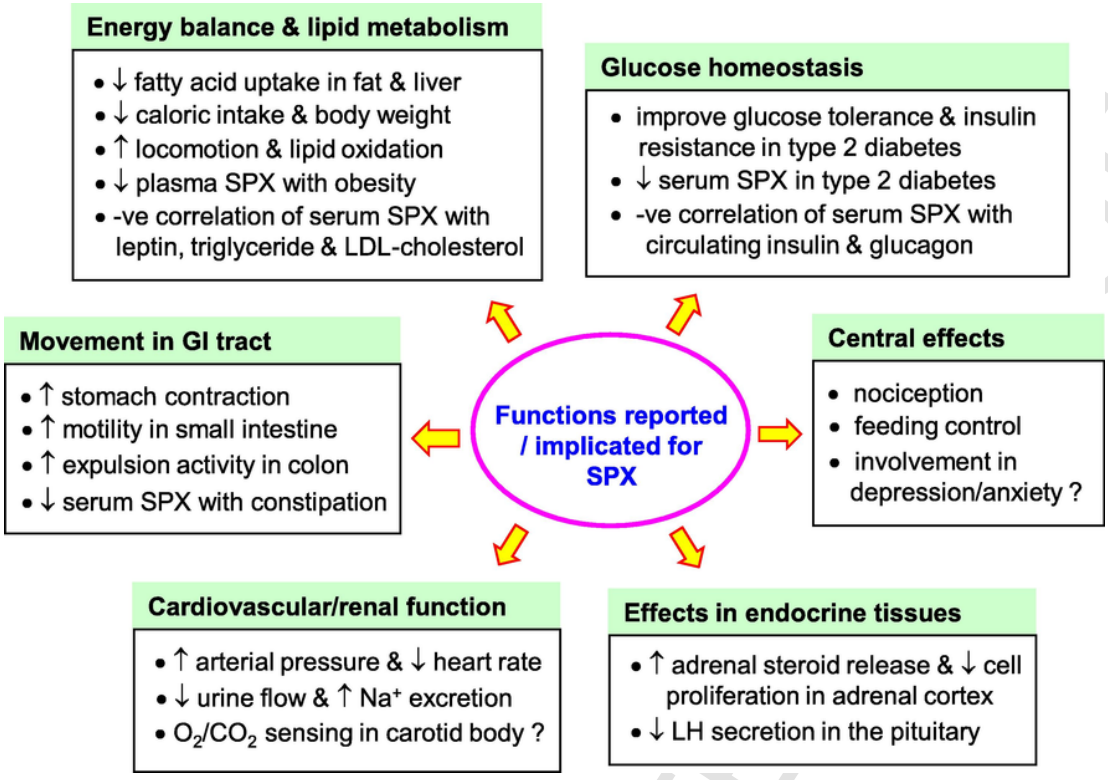

Fig. 3. Biological functions confirmed or implicated for SPX by in vivo/in vitro studies in animal models or by correlation analysis in human subjects with/without pathophysiological conditions, including obesity, diabetes and/or functional constipation (refer to the text for details).

SPX signals have been reported in the GI tract with notable levels of SPX immunoreactivity detected in the mucosal layer of the stomach, small intestine and colon (Gu et al., 2015) and a drop in plasma SPX is commonly observed in patient with functional constipation (Lin et al., 2015). These findings, taken together, suggest that SPX produced within the GI tract may play a role in the control of gut movement/stomach contraction after food intake and may have therapeutic value for GI diseases with motility disorders.

In mammals, Gal is well-documented to have antinociceptive action (Xu et al., 2010) and the effect can be exerted via its local expression in the spinal cord (Xu et al., 2012) or in brain areas including the hypothalamus (Amorim et al., 2015), arcuate nucleus (Gu et al., 2007), nucleus accumbens (Duan et al., 2015) and central nucleus of amygdala (Jin et al., 2010). Using a pharmacological approach, the central actions of Gal for nociception have been confirmed to be mediated via GalR1 (Fu et al., 2011) and GalR2 activation (Zhang et al., 2017, 2015). In the rat, similar to Gal, SPX is widely expressed in different brain areas (Porzionato et al., 2010) and ICV injection of SPX has been shown to trigger antinociceptive activity as revealed by a warm water tail withdrawal assay (Toll et al., 2012). Given that the effect of SPX is not sensitive to the blockade by opioid antagonists (e.g., naloxone), the opioidergic system within the brain is not likely to be involved in SPX-induced analgesia (Toll et al., 2012). Using formalin injection into the hind paw of female rat followed by scoring of different levels of pain sensitivity, the antinociceptic effect of SPX has been confirmed by bilateral injection of SPX into CA3 area of the hippocampus (Pizeh and Taherianfard, 2014). In a recent study using a similar approach, SPX-induced analgesia in ovariectomized rat could be notably enhanced with co-treatment with progesterone, suggesting that sex steroid and SPX can act in a synergistic manner in modulating pain sensitivity via central action (Moazen et al., 2017). Although the antinociceptive action of SPX has been confirmed in animal model (e.g., rat), the receptor specificity for SPX as well as its functional interaction with Gal in pain detection is still unknown. Of note, differential expression of SPX in different brain areas, with up-regulation in the hippocampus and striatum but down-regulation in the hypothalamus, has been reported in the rat with long-term treatment of escitalopram, a serotonin reuptake inhibitor commonly used for depression and anxiety (Palasz et al., 2016), which has raised the concern for a possible link of SPX with mood disorder/related psychiatric diseases. The idea is also supported by the anxiolytic effect reported recently for the newly developed SPX-based GalR2 agonist dN1-Qu demonstrated in the mouse model (Reyes-Alcaraz et al., 2016).

Besides the GI tract and central nervous system, SPX expression can also be found in a wide range of endocrine tissues (Gu et al. 2015). Apparently, the adrenal gland represents the major endocrine organ with a high level of SPX expression, both at the transcript and protein levels (Gu et al., 2015; Mirabeau et al., 2007). Based on immunohistochemcial staining in the adrenal gland of the rat (Porzionato et al., 2010) and human (Gu et al., 2015), SPX can be detected mainly in the adrenal cortex (including the glomerulosa and fasciculate/reticularis layers) with low level of expression in the medulla region. In rat adrenocortical cells, SPX treatment is effective in triggering a mild/weak induction of aldosterone and corticosterone secretion with concurrent inhibition of cell proliferation (Rucinski et al., 2010). In the same study, SPX expression in adrenal cortex can be up-regulated by dexamethasone treatment or by adrenal enucleation, but the opposite is true with stimulation by ACTH. These findings suggest that SPX is produced locally in the adrenal gland under hormonal influence and may play a role in steroid production and adrenal regeneration after injury. It is also worth mentioning that ICV

injection of SPX in the rat is known to elevate arterial pressure with a concurrent drop in heart rate and urine flow (Toll et al., 2012). Although the physiological relevance and the mechanisms involved in these findings are still unknown, the possible role of SPX as a central regulator for cardiovascular/renal function cannot be excluded. Furthermore, SPX signals have been detected in the rat and human carotid body (especially in the type I glomic cells) and SPX expression in the carotid body, both at transcript and protein levels, can be up-regulated in neonatal rat by exposure to hypoxic condition (Porzionato et al., 2012), implying that SPX expression may be associated with O_2/CO_2 sensing in mammalian species.

5. . Emerging functions of spexin in fish models

To date, the research on SPX is largely restricted to mammals and the comparative aspects of SPX, especially in lower vertebrates, are still lagging behind. By data mining and molecular cloning, SPX sequences have been reported in fish models including zebrafish (Sonmez et al., 2009), goldfish (Liu et al., 2013), ya-fish (Wu et al., 2016), grouper (Li et al., 2016), medaka and fugu (Kim et al., 2014). Of note, the second form of SPX, namely SPX_2, has also been identified in zebrafish and medaka but not in fugu (Kim et al., 2014) but its biological function is still unclear in fish models. Based on the studies in goldfish, SPX has been shown to play a role in reproduction by altering the functionality of the hypothalamo-pituitary-gonadal axis (Liu et al., 2013) (Fig. 4A). In female goldfish, SPX treat-

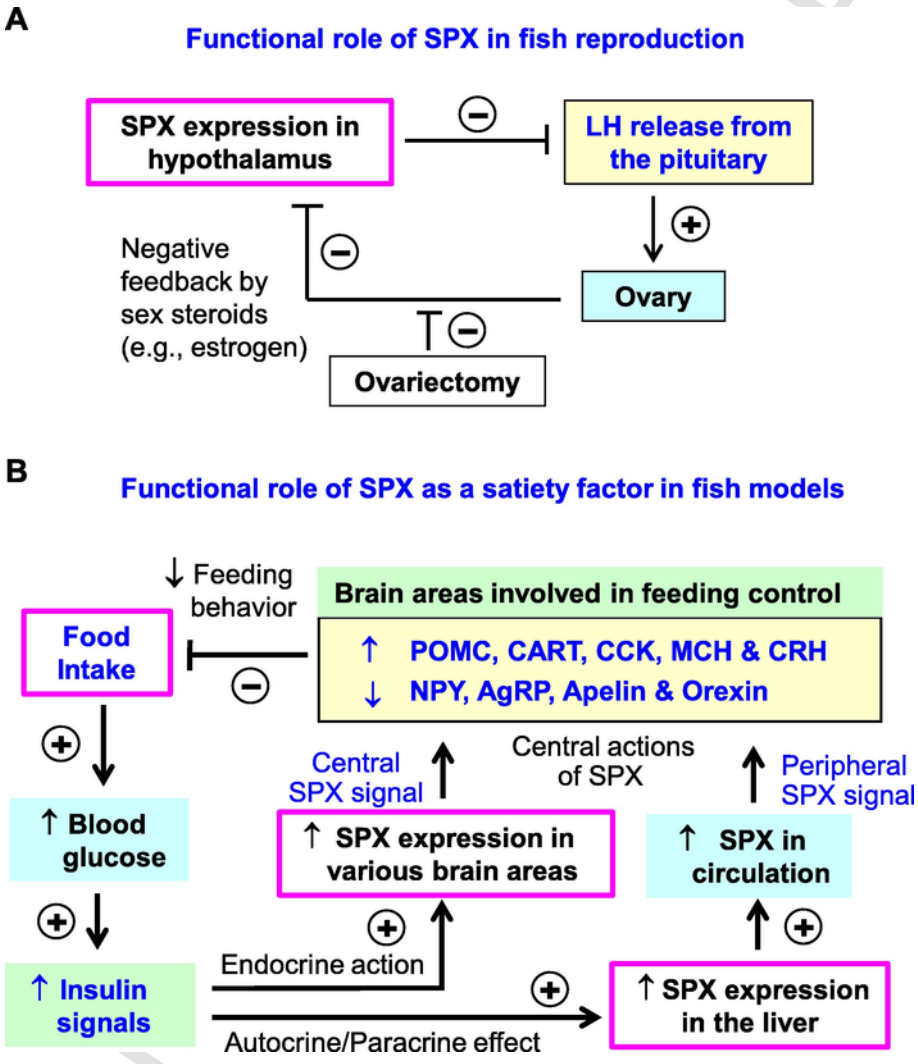

Fig. 4. Functional role of SPX in reproduction and feeding control in fish models. (A) Involvement of SPX in gonadal feedback on LH secretion. In female goldfish, SPX treatment can inhibit LH secretion via direct action acting at the pituitary level. Meanwhile, sex steroids, especially estrogen, produced by the ovary can suppress SPX expression in the hypothalamus and this effect can be blocked by ovariectomy, suggesting that SPX may act as a novel component for gonadal feedback via sex steroids on the hypothalamo-pituitary axis. (B) Functional role of SPX as a satiety factor in fish models (e.g., goldfish, ya fish, grouper and zebrafish). In fish models, especially in goldfish, food intake is known to stimulate SPX expression in brain areas involved in appetite control, including the hypothalamus, telencephalon and optic tectum. The central expression of SPX has been confirmed to be the result of insulin signals (mainly from the pancreas) caused by the rise in blood glucose after feeding. Besides the endocrine component of insulin, insulin can also be produced in the fish liver (e.g., in goldfish) under the influence of blood glucose, which can act in an autocrine/paracrine manner to induce SPX expression and secretion at the hepatic level. The SPX output from the liver constitutes a circulating source of SPX, which may act together with the central expression of SPX to differentially regulate orexigenic (e.g., NPY, AgRP, apelin & orexin) and anorexigenic signals (e.g., POMC, CART, CCK, MCH & CRH) in different brain areas to suppress feeding behaviour and food consumption in fish models.

ment is effective in inhibiting LH secretion, both in vivo and in vitro, and basal level of SPX expression in the hypothalamus can be gradually reduced with sexual maturation during the reproductive cycle. In the same model, SPX mRNA level in the hypothalamus can be up-regulated by ovariectomy and this effect can be blocked by estrogen replacement (Liu et al., 2013). These finding, as a whole, suggest that SPX expression in the hypothalamus may be involved in gonadal feedback on LH regulation. In a similar study with grouper, although a gradual drop in SPX expression can still be noted in the hypothalamus during sexual maturation, SPX treatment is not effective in altering LHβ and FSHβ mRNA levels in the pituitary (Li et al., 2016). In a recent report in zebrafish with SPX knockout, the *spx*$^{-/-}$ mutant is fertile with no abnormality in puberty onset or gamete maturation in the testes and ovary (Zheng et al., 2017), suggesting that the reproductive function of SPX may be species-specific in fish models.

In our functional studies with goldfish, food intake was shown to stimulate SPX expression in the telencephalon, hypothalamus and optic tectum, while brain injection of SPX not only could suppress food consumption and feeding behavior but also induce rapid expression of anorexigenic factors (e.g., CART, POMC, CCK & MCH) with parallel drop in orexigenic signals (e.g., NPY, AgRP & apelin) in the brain areas with SPX responses (Wong et al., 2013). Our findings on the postprandial rise in SPX expression in the brain and differential regulation of feeding signals in the hypothalamus by SPX have also been confirmed by similar experiments in ya-fish (Wu et al., 2016) and grouper (Li et al., 2016), respectively. In the recent study in zebrafish with SPX knockout, the *spx*$^{-/-}$ mutant has been reported to exhibit elevated expression of AgRP in the hypothalamus with a higher level of food consumption. In the same study, intracranial injection of SPX in the wild type is also effective in reducing AgRP expression in the hypothalamus (Zheng et al., 2017). These findings, together with ours, support the idea that SPX expression can be induced by feeding, which can then trigger the satiation response via central actions to differentially regulate orexigenic and anorexigenic signals expressed in brain areas related to appetite control in fish models (Fig. 4B). In our recent study with goldfish, the insulin signal caused by glucose uptake after feeding was confirmed to be the functional link between food intake and SPX expression (Ma et al., 2017). In this case, insulin by acting as an endocrine signal could mediate the stimulatory effect caused by a rise in blood glucose to induce SPX expression in the telencephalon, hypothalamus and optic tectum. Meanwhile, insulin was also found to be expressed in the liver and act in an autocrine/paracrine manner to induce SPX synthesis and secretion at the hepatic level, which could lead to a rise in plasma SPX level and contribute to the central regulation of feeding signals in the goldfish. Based on our study, besides acting through insulin receptor coupled to $P_{38}{}^{MAPK}$ and PI3K/Akt signaling pathways, insulin-induced SPX expression was also partly mediated through IGF-I receptor activation both in the brain as well as in the liver (Ma et al., 2017), which raises the possibility that SPX regulation may also be linked with the GH/IGF-I axis in fish models.

6. . Concluding remarks and future perspectives

Based on the recent studies in mammals as well as in fish models, evidence starts to emerge for the pleotropic functions of SPX, which are consistent with its wide range of tissue distribution observed in different vertebrate classes. SPX can exert its biological actions by (i) serving as an endocrine signal in systemic circulation and (ii) acting as a neurotransmitter/neuromodulator within the central nervous system. The endocrine functions of SPX can be related to its effects on feeding control and energy balance as well as its potential role in lipid and glucose homeostasis, whereas its central actions can be related to its involvement in nociception/mood disorders as well as its regulatory actions in brain areas for appetite control and reproductive functions via the hypothalamo-pituitary axis. Of note, GalR2 and GalR3 have been identified as the cognate receptors for SPX (Kim et al., 2014) and tissue distribution of GalR2/GalR3, e.g., in the brain (Barreda-Gomez et al., 2005; Lu et al., 2005) and GI tract (Anselmi et al., 2005; Arciszewski et al., 2008), are also found to overlap with that reported for SPX expression (e.g., in the rat). The possibility of SPX to act as an autocrine/paracrine factor at tissue level cannot be excluded, especially for its functions in the adrenal cortex (Rucinski et al., 2010) as well as in stomach contraction/gut motility (Lin et al., 2015; Mirabeau et al., 2007). In mammals, the functional coupling of GalR2 with $G_{q/11}$ and GalR3 with G_i for differential regulation of cAMP/PKA-, Ca^{2+}-, MAPK- and PI3K/Akt-dependent cascades are well-documented for the biological actions of Gal (Lang et al., 2015; Sipkova et al., 2017). However, except for a single report for SPX-induced gut motility in the mouse via Ca^{2+} entry through L-type voltage-sensitive Ca^{2+} channels (Lin et al., 2015), no information is available regarding the signal transduction involved in the biological actions of SPX. Judging from the fact that two forms of GalR2, GalR2a and GalR2b, have been identified in fish models and shown to have overlapping and yet distinct ligand selectivity compared to the mammalian GalR2 (Kim et al., 2014), their post-receptor signaling related to the biological functions of SPX_1 and SPX_2 identified in non-mammalian species for sure are interesting questions waiting to be clarified. Since SPX has been implicated in weight control in animal model with dietary-induced obesity (Walewski et al., 2014) and glucose homeostasis in patients with type 2 diabetes (Gu et al., 2015), its potential role as a therapeutic target will be useful for future development of new technology for treatment of obesity and metabolic disorders.

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Disclosure

The authors have nothing to disclose for potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ygcen.2018.01.015.

References

Agha, M., Agha, R., 2017. The rising prevalence of obesity: Part A. Impact on public health. Int. J. Surg. Oncol. (N Y) 2, e17.

Amorim, D., Viisanen, H., Wei, H., Almeida, A., Pertovaara, A., Pinto-Ribeiro, F., 2015. Galanin-mediated behavioural hyperalgesia from the dorsomedial nucleus of the hypothalamus involves two iIndependent descending pronociceptive pathways. PLoS One 10, e0142919.

Anselmi, L., Stella Jr., S.L., Lakhter, A., Hirano, A., Tonini, M., Sternini, C., 2005. Galanin receptors in the rat gastrointestinal tract. Neuropeptides 39, 349–352.

Arciszewski, M.B., Barabasz, S., Calka, J., 2008. Immunohistochemical localization of galanin receptors (GAL-R1, GAL-R2, and GAL-R3) on myenteric neurons from the sheep and dog stomach. Ann. Anat. 190, 360–367.

Barreda-Gomez, G., Giralt, M.T., Rodriguez-Puertas, R., 2005. G protein-coupled galanin receptor distribution in the rat central nervous system. Neuropeptides 39, 153–156.

Duan, H., Zhang, Y., Zhang, X.M., Xu, H.H., Shu, J., Xu, S.L., 2015. Antinociceptive roles of galanin receptor 1 in nucleus accumbens of rats in a model of neuropathic pain. J. Neurosci. Res. 93, 1542–1551.

Fu, L.B., Wang, X.B., Jiao, S., Wu, X., Yu, L.C., 2011. Antinociceptive effects of intracerebro-ventricular injection of the galanin receptor 1 agonist M 617 in rats. Neurosci. Lett. 491, 174–176.

Ge, J.F., Walewski, J.L., Anglade, D., Berk, P.D., 2016. Regulation of hepatocellular fatty acid uptake in mouse models of fatty liver disease with and without functional leptin signaling: roles of NfKB and SREBP-1C and the effects of spexin. Semin. Liver Dis. 36, 360–372.

Gu, L., Ma, Y.H., Gu, M.Y., Zhang, Y., Yan, S., Li, N., Wang, Y.F., Ding, X.Y., Yin, J.J., Fan, N.G., Peng, Y.D., 2015. Spexin peptide is expressed in human endocrine and epithelial tissues and reduced after glucose load in type 2 diabetes. Peptides 71, 232–239.

Gu, X.L., Sun, Y.G., Yu, L.C., 2007. Involvement of galanin in nociceptive regulation in the arcuate nucleus of hypothalamus in rats with mononeuropathy. Behav. Brain Res. 179, 331–335.

Hodges, S.K., Teague, A.M., Dasari, P.S., Short, K.R., 2017. Effect of obesity and type 2 diabetes, and glucose ingestion on circulating spexin concentration in adolescents. Pediatr Diabetes https://doi.org/10.1111/pedi.12549.

Jin, W.Y., Liu, Z., Liu, D., Yu, L.C., 2010. Antinociceptive effects of galanin in the central nucleus of amygdala of rats, an involvement of opioid receptors. Brain Res. 1320, 16–21.

Kim, D.K., Yun, S., Son, G.H., Hwang, J.I., Park, C.R., Kim, J.I., Kim, K., Vaudry, H., Seong, J.Y., 2014. Coevolution of the spexin/galanin/kisspeptin family: spexin activates galanin receptor type II and III. Endocrinology 155, 1864–1873.

Kolodziejskii, P.A., Pruszynska-Oszmalek, E., Korek, E., Sassek, M., Szczepankiewicz, D., Kaczmarek, P., Nogowski, L., Mackowiak, P., Nowak, K.W., Krauss, H., Strowski, M.Z., 2017. Serum levels of spexin and kisspeptin negatively correlate with obesity and insulin resistance in women. Physiol Res.[Epud ahead of print].

Kumar, S., Hossain, J., Nader, N., Aguirre, R., Sriram, S., Balagopal, P.B., 2016. Decreased circulating levels of spexin in obese children. J Clin. Endocrinol. Metab. 101, 2931–2936.

Kumar, S., Hossain, M.J., Javed, A., Kullo, I.J., Balagopal, P.B., 2017. Relationship of circulating spexin with markers of cardiovascular disease: a pilot study in adolescents with obesity. Pediatr. Obes. https://doi.org/10.1111/ijpo.12249.

Lang, R., Gundlach, A.L., Holmes, F.E., Hobson, S.A., Wynick, D., Hokfelt, T., Kofler, B., 2015. Physiology, signaling, and pharmacology of galanin peptides and receptors: three decades of emerging diversity. Pharmacol. Rev. 67, 118–175.

Li, S., Liu, Q., Xiao, L., Chen, H., Li, G., Zhang, Y., Lin, H., 2016. Molecular cloning and functional characterization of spexin in orange-spotted grouper (Epinephelus coioides). Comp. Biochem. Physiol. B Biochem. Mol. Biol. 196, 85–91.

Lin, C.Y., Zhang, M., Huang, T., Yang, L.L., Fu, H.B., Zhao, L., Zhong, L.D.D., Mu, H.X., Shi, X.K., Leung, C.F.P., Fan, B.M., Jiang, M., Lu, A.P., Zhu, L.X., Bian, Z.X., 2015. Spexin enhances bowel movement through activating L-type voltage-dependent calcium channel via galanin receptor 2 in mice. Sci. Rep. 5, 12095. https://doi.org/10.1038/srep12095.

Liu, Y., Li, S., Qi, X., Zhou, W., Liu, X., Lin, H., Zhang, Y., Cheng, C.H., 2013. A novel neuropeptide in suppressing luteinizing hormone release in goldfish Carassius auratus. Mol. Cell Endocrinol. 374, 65–72.

Lu, X., Mazarati, A., Sanna, P., Shinmei, S., Bartfai, T., 2005. Distribution and differential regulation of galanin receptor subtypes in rat brain: effects of seizure activity. Neuropeptides 39, 147–152.

Ma, A.I., He, M.L., Bai, J., Wong, M.K.H., Ko, W.K.W., Wong, A.O.L., 2017. Dual role of insulin in spexin regulation: functional link between food intake and spexin expression in a fish model. Endocrinology 158, 560–577.

Merkler, D.J., 1994. C-terminal amidated peptides: production by the in vitro enzymatic amidation of glycine-extended peptides and the importance of the amide to bioactivity. Enzyme Microb. Technol. 16, 450–456.

Mirabeau, O., Perlas, E., Severini, C., Audero, E., Gascuel, O., Possenti, R., Birney, E., Rosenthal, N., Gross, C., 2007. Identification of novel peptide hormones in the human proteome by hidden Markov model screening. Genome Res. 17, 320–327.

Moazen, P., Taherianfard, M., Ahmadi Soleimani, M., Norozpor, M., 2017. Synergistic effect of spexin and progesterone on pain sensitivity attenuation in ovariectomized rats. Clin. Exp. Pharmacol. Physiol. https://doi.org/10.1111/1440-1681.12862.

Palasz, A., Suszka-Switek, A., Filipczyk, L., Bogus, K., Rojczyk, E., Worthington, J., Krzystanek, M., Wiaderkiewicz, R., 2016. Escitalopram affects spexin expression in the rat hypothalamus, hippocampus and striatum. Pharmacol. Rep. 68, 1326–1331.

Pizeh, L., Taherianfard, M., 2014. Effect of intra hippocampal CA1 injection of spexin on pain sensitivity in female rat. Bull. Env. Pharmacol. Life Sci. 3, 71–74.

Porzionato, A., Rucinski, M., Macchi, V., Stecco, C., Malendowicz, L.K., De Caro, R., 2010. Spexin expression in normal rat tissues. J. Histochem. Cytochem. 58, 825–837.

Porzionato, A., Rucinski, M., Macchi, V., Stecco, C., Sarasin, G., Sfriso, M.M., Di Giulio, C., Malendowicz, L.K., De Caro, R., 2012. Spexin is expressed in the carotid body and is upregulated by postnatal hyperoxia exposure. Adv. Exp. Med. Biol. 758, 207–213.

Reyes-Alcaraz, A., Lee, Y.N., Son, G.H., Kim, N.H., Kim, D.K., Yun, S., Kim, D.H., Hwang, J.I., Seong, J.Y., 2016. Development of spexin-based human galanin receptor type II-specific agonists with increased stability in serum and anxiolytic effect in mice. Sci. Rep. 6, 21453.

Rucinski, M., Porzionato, A., Ziolkowska, A., Szyszka, M., Macchi, V., De Caro, R., Malendowicz, L.K., 2010. Expression of the spexin gene in the rat adrenal gland and evidences suggesting that spexin inhibits adrenocortical cell proliferation. Peptides 31, 676–682.

Sipkova, J., Kramarikova, I., Hynie, S., Klenerova, V., 2017. The galanin and galanin receptor subtypes, its regulatory role in the biological and pathological functions. Physiol. Res. 66, 729–740.

Sonmez, K., Zaveri, N.T., Kerman, I.A., Burke, S., Neal, C.R., Xie, X., Watson, S.J., Toll, L., 2009. Evolutionary sequence modeling for discovery of peptide hormones. PLoS Comput. Biol. 5, e1000258.

Toll, L., Khroyan, T.V., Sonmez, K., Ozawa, A., Lindberg, I., McLaughlin, J.P., Eans, S.O., Shahien, A.A., Kapusta, D.R., 2012. Peptides derived from the prohormone proNPQ/spexin are potent central modulators of cardiovascular and renal function and nociception. FASEB J. 26, 947–954.

Walewski, J.L., Ge, F., Gagner, M., Inabnet, W.B., Pomp, A., Branch, A.D., Berk, P.D., 2010. Adipocyte accumulation of long-chain fatty acids in obesity is multifactorial, resulting from increased fatty acid uptake and decreased activity of genes involved in fat utilization. Obes. Surg. 20, 93–107.

Walewski, J.L., Ge, F., Lobdell, H.T., Levin, N., Schwartz, G.J., Vasselli, J.R., Pomp, A., Dakin, G., Berk, P.D., 2014. Spexin is a novel human peptide that reduces adipocyte uptake of long chain fatty acids and causes weight loss in rodents with diet-induced obesity. Obesity (Silver Spring) 22, 1643–1652.

Wan, B., Wang, X.-R., Zhou, Y.-B., Zhang, X., Huo, K., Han, Z.-G., 2010. C12ORF39, a novel secreted protein with a typical amidation processing signal. Biosci. Rep. 30, 1–10.

Wong, M.K.H., Sze, K.H., Chen, T., Cho, C.K., Law, H.C.H., Chu, I.K., Wong, A.O.L., 2013. Goldfish spexin: solution structure and novel function as a satiety factor in feeding control. Am. J. Physiol. Endocrinol. Metabol. 305, E348–E366.

Wu, H., Lin, F., Chen, H., Liu, J., Gao, Y., Zhang, X., Hao, J., Chen, D., Yuan, D., Wang, T., Li, Z., 2016. Ya-fish (Schizothorax prenanti) spexin: identification, tissue distribution and mRNA expression responses to periprandial and fasting. Fish Physiol. Biochem. 42, 39–49.

Xu, X., Yang, X., Zhang, P., Chen, X., Liu, H., Li, Z., 2012. Effects of exogenous galanin on neuropathic pain state and change of galanin and its receptors in DRG and SDH after sciatic nerve-pinch injury in rat. PLoS One 7, e37621.

Xu, X.J., Hokfelt, T., Wiesenfeld-Hallin, Z., 2010. Galanin and spinal pain mechanisms: past, present, and future. EXS 102, 39–50.

Yant, L., Bomblies, K., 2015. Genome management and mismanagement: cell-level opportunities and challenges of whole-genome duplication. Genes Dev. 29, 2405–2419.

Zhang, M.L., Wang, H.B., Fu, F.H., Yu, L.C., 2017. Involvement of galanin and galanin receptor 2 in nociceptive modulation in anterior cingulate cortex of normal rats and rats with mononeuropathy. Sci. Rep. 7, 45930.

Zhang, X.Y., Zhang, Y.M., Zhang, M.L., Yu, L.C., 2015. Involvement of galanin receptor 2 and CaMKII in galanin-induced antinociception in periaqueductal grey of rats. Neurosci. Lett. 604, 124–127.

Zheng, B., Li, S., Liu, Y., Li, Y., Chen, H., Tang, H., Liu, X., Lin, H., Zhang, Y., Cheng, C.H.K., 2017. Spexin suppress food intake in zebrafish: evidence from gene knockout study. Sci. Rep. 7, 14643.