

Role of developmental factors in hypothalamic function

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The hypothalamus is a brain region which regulates homeostasis by mediating endocrine, autonomic and behavioral functions. It is comprised of several nuclei containing distinct neuronal populations producing neuropeptides and neurotransmitters that regulate fundamental body functions including temperature and metabolic rate, thirst and hunger, sexual behavior and reproduction, circadian rhythm, and emotional responses. The identity, number and connectivity of these neuronal populations are established during the organism's development and are of crucial importance for normal hypothalamic function. Studies have suggested that developmental abnormalities in specific hypothalamic circuits can lead to obesity, sleep disorders, anxiety, depression and autism. At the molecular level, the development of the hypothalamus is regulated by transcription factors (TF), secreted growth factors, neuropeptides and their receptors. Recent studies in zebrafish and mouse have demonstrated that some of these molecules maintain their expression in the adult brain and subsequently play a role in the physiological functions that are regulated by hypothalamic neurons. Here, we summarize the involvement of some of the key developmental factors in hypothalamic development and function by focusing on the mouse and zebrafish genetic model organisms.

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Introduction

The hypothalamus is a key regulator of homeostasis in animals. It does so by integrating internal and external sensory signals, processing them, then exerting regulatory autonomic signals and neuroendocrine releasing peptides to maintain homeostasis (Pearson and Placzek, 2013). Hypothalamus-related neuropeptides were identified in ganglions of lower evolutionary animals such as corals and clams (Twan et al., 2006; Takayanagi and Onaka, 2010) and ontogenetic conservation of hypothalamus-related structures in the brains of Annelids and zebrafish has been demonstrated (Tessmar-Raible et al., 2007). In vertebrates, the hypothalamus resides ventrally to the thalamus, dorsally to the anterior pituitary and is structurally composed of several nuclei of interconnected cell populations. Each nucleus contains several neuronal types, and these work in an orchestrated manner within and between nuclei to regulate physiological functions including metabolism, water balance, satiety, reproductive physiology, circadian rhythm, and emotional responses (Machluf et al., 2011; Pearson and Placzek, 2013). Developmental abnormalities of the hypothalamus are associated with alterations in body growth and sexual development leading to adverse physiological and neurological conditions such as infertility, depression, chronic stress, autism and obesity (Michaud, 2001; Swaab, 2004; Silveira et al., 2010). Moreover, interaction of hypothalamic neurons with neighboring neuronal, astroglial and endothelial cells is highly important for sensing

blood-borne hormones and metabolites. Failure to generate these interactions might lead to impairment in negative feedback signals, release of trophic neuropeptides and abnormalities in the structure of the neurohypophysial portal system (de Seranno et al., 2010; Gutnick et al., 2011).

Major efforts in recent years have been focused on the roles of hypothalamic transcription factors (TF) and signaling molecules, as well as identification and characterization of the molecular and biochemical mechanisms they regulate during the organization of distinct hypothalamic regions, their differentiation, and circuit connectivity (reviewed by Machluf et al., 2011; Pearson and Placzek, 2013). Interestingly, some of these essential developmental factors are also expressed in the mature hypothalamus (Bardet et al., 2008; Tolson et al., 2010). A few studies have directly addressed the non-developmental role of these factors in the proper functioning of mature hypothalamic nuclei, suggesting that proper regulation by these factors is essential for normal activation of the hypothalamus. These findings support the hypothesis that developmental and post-developmental impairment of these components may lead to hypothalamus-related disorders, such as infertility, obesity, depression and chronic stress.

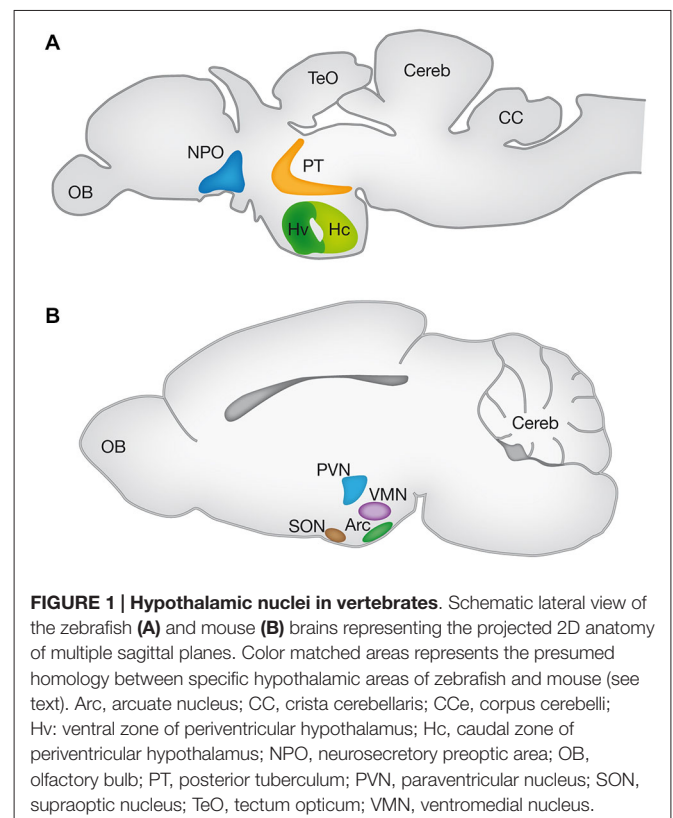
Here, we summarize some key developmental factors involved in hypothalamic development and function. The periventricular zone of the hypothalamus contains several nuclei including the paraventricular nucleus (PVN), arcuate nucleus (Arc), supra-chiasmatic nucleus, and the anterior periventricular nucleus (aPV; Szarek et al., 2010). These hypothalamic areas are well characterized for their physiological roles, allowing the investigation of both developmental and functional regulation. Hence, we have focused our review mainly on TFs expressed in this brain region. To gain better insights on the molecular mechanisms conveyed by these TFs, we mainly focused on the mouse and zebrafish genetic models, in which specific genetic perturbations have unveiled the functions of these factors. Lastly, we discuss the possible link between factors that regulate hypothalamic development to neurodevelopmental disorders that disrupt both physiological and psychological homeostasis.

Comparative Neuroanatomy of the Hypothalamus

In recent years, several publications emphasized the importance and relevance of non-mammalian model organisms for the study of hypothalamic development with zebrafish as the prominently utilized model (reviewed in Machluf et al., 2011; Pearson and Placzek, 2013; Wircer et al., in press). The general organization of the vertebrate brain is evolutionarily conserved, however several events during tetrapod and mammalian evolution led to neuroanatomical changes (Suárez et al., 2014). Importantly, it has been shown that key genetic factors driving the patterning and specification of major hypothalamic nuclei are evolutionarily conserved (Tessmar-Raible et al., 2007). Hence, understanding the neuroanatomical homology of the various hypothalamic nuclei is important in order to integrate the information, which has been obtained from various model organisms.

One approach for comparative identification of the hypothalamic nuclei is based on expression patterns of mRNAs and proteins of evolutionarily conserved TFs and neuropeptides. In this regards, we will discuss findings from two prevalent genetic models namely the mouse and zebrafish, focusing on the Arc, PVN and supraoptic nuclei (SON) of the mouse hypothalamus and the ventral zone of the periventricular hypothalamus (Hv), neurosecretory preoptic area (NPO) and ventral posterior tuberculum (vPT) of the zebrafish. Schematic localization of these nuclei in the adult brains of these animals is illustrated in **Figure 1**.

Several studies have demonstrated that the Hv of the zebrafish (also known as nucleus lateralis tuberis; NLT) and the mammalian Arc are homologous as both nuclei express Neurokinin B (Ramaswamy et al., 2010; Biran et al., 2012; Ogawa et al., 2012), Kisspeptin (Ramaswamy et al., 2010; Servili et al., 2011; Ogawa et al., 2012), GHRH (Farhy and Veldhuis, 2004; Castro et al., 2009), α MSH and AgRP (Forlano and Cone, 2007; Guzmán-Ruiz et al., 2014). The NPO of the fish and its homologous mammalian PVN were shown to express oxytocin (OXT; Wang and Lufkin, 2000; Goodson et al., 2003; Unger and Glasgow, 2003; Löhr et al., 2009; Gutnick et al., 2011; Fernandes et al., 2013; Herget et al., 2014), Arginine vasopressin (AVP; Wang and Lufkin, 2000; Eaton et al., 2008; Löhr et al., 2009; Fernandes et al., 2013; Herget et al., 2014), Corticotropin-releasing hormone (CRH; Wang and Lufkin, 2000; Löhr et al., 2009; Amir-Zilberstein et al., 2012; Fernandes et al., 2013; Herget et al., 2014) and Somatostatin

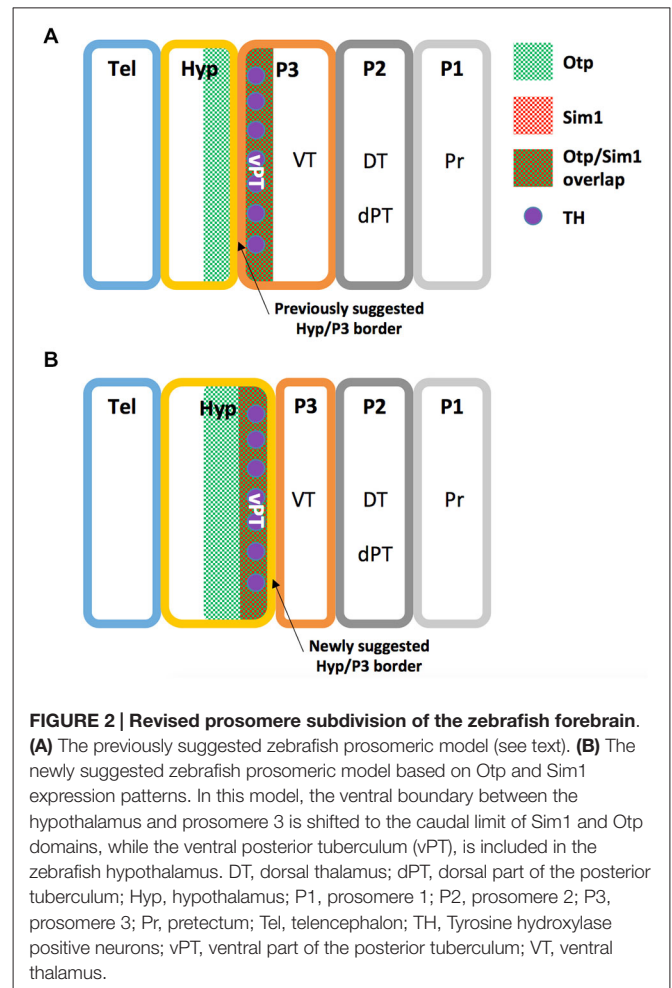


(SST; Wang and Lufkin, 2000; Blechman et al., 2007; Russek-Blum et al., 2008; Löhr et al., 2009; Fernandes et al., 2013; Herget et al., 2014).

A recent study illustrates the high homology between the zebrafish NPO and the mammalian PVN by both peptidergic and specific NPO TFs (Herget et al., 2014). However, no piscine hypothalamic nucleus is recognized as homologous to the mammalian SON, a key hypothalamic nucleus expressing the neurohypophyseal hormones OXT and AVP (Wircer et al., in press). Moreover, the piscine NPO was recently suggested as the common evolutionary ancestor of the vertebrate magnocellular neuronal cluster, which later anatomically partitions to generate the PVN and SON in mammals (Gutnick et al., 2011; Herget et al., 2014; Knobloch and Grinevich, 2014). However, since mammalian PVN and SON neurons differ in their origin from the preoptic area neurons (Altman and Bayer, 1978a,b; Markakis, 2002), this hypothesis should be carefully considered. Interestingly, the PT in fish is another brain region that is not considered a classical hypothalamic region (Wullimann and Rink, 2001, 2002), but a careful look at the literature might suggest otherwise. Firstly, the periventricular zone of PT (vPT) was shown to express neuropeptides which are characteristic of the mammalian periventricular hypothalamus such as AVP (Wang and Lufkin, 2000; Hatae et al., 2001; Goodson et al., 2003; Eaton et al., 2008; Fernandes et al., 2013), CRH (Wang and Lufkin, 2000; Amir-Zilberstein et al., 2012; Fernandes et al., 2013) and Neurokinin B (Hatae et al., 2001; Biran et al., 2012). Secondly, catecholaminergic (tyrosine hydroxylase expressing) cells of the vPT were suggested to be homologous to hypothalamic group A11 of dopaminergic cells (Ryu et al., 2007; Löhr et al., 2009; Filippi et al., 2014). Notably, Puelles and Rubenstein define the boundary between the caudal hypothalamus and diencephalic prosomere 3 in rodents by the expression of several genes, including the TFs Single minded (Sim1) and Orthopedia (Otp), which are expressed exclusively in the hypothalamus (Puelles and Rubenstein, 1993, 2003). As both Sim1 and Otp are expressed in the zebrafish vPT (Borodovsky et al., 2009; Löhr et al., 2009; Fernandes et al., 2013) we propose a revised prosomere subdivision of the zebrafish forebrain (Figure 2), in which the boundary between the caudal hypothalamus and prosomere 3 is shifted to the caudal limit of Sim1 and Otp domains, so that the vPT is regarded as part of the teleostian hypothalamus. Future comparative gene expression analyses, fate-mapping experiments and other comparative anatomy experiments are required to further establish this refined model.

Transcription Factors Regulating the Development of the Vertebrate Hypothalamus

The hypothalamus contains anatomical partitioning and its various neuronal cell populations form elaborate connectivity with virtually all parts of the nervous system. This raises many questions regarding the mechanisms that underlie the development of hypothalamic brain nuclei and the



specification of the neuronal populations that inhabit the hypothalamus.

Otp–

The homeodomain-containing TF Otp is well conserved across species. The deduced amino acid sequence of the homeodomain of the human protein is 99% homologous to the mouse Otp, and demonstrates high degree of conservation when compared to sea urchin, drosophila (Lin et al., 1999) and planaria (Umesono et al., 1997). Additionally, the existence of several evolutionarily conserved non-coding sequences (ECR) was recently demonstrated in the Otp promoter by Gutierrez-Triana et al. (2014). Using zebrafish as their model, the authors have further demonstrated that OtpaECR6 specifically regulates the expression of Otp in the NPO of the zebrafish (Gutierrez-Triana et al., 2014). Taken together, this conservation suggests an evolutionarily conserved functional role for Otp in vertebrates. Otp is expressed in conserved hypothalamic domains (Simeone et al., 1994; Bardet et al., 2008; Del Giacco et al., 2008), where it plays an important role in the differentiation of several neurohormone-secreting nuclei including the aPV, PVN, SON, Arc and ventromedial nucleus (VMN; Acampora et al., 1999; Blechman et al., 2007; Eaton

et al., 2008). In the zebrafish, the expression of *Otp* in the NPO and PT is regulated by the zinc-finger-containing TF *Fezf2* (Blechman et al., 2007; Machluf et al., 2011; Yang et al., 2012; Wolf and Ryu, 2013). Non-hypothalamic expression of *Otp* is also detected in the medial amygdaloid nucleus (MeA), hindbrain and spinal cord (Simeone et al., 1994; Acampora et al., 1999). Importantly, *Otp* positive neurons inhabiting the murine MeA are of diencephalic origin. These neurons are generated in the hypothalamus and migrate during brain development through the diencephalic-mesencephalic junction into their final position in the MeA (García-Moreno et al., 2010).

It has been demonstrated that *Otp* is crucial for proper development of diencephalic dopaminergic neurons in zebrafish and mouse. Zebrafish embryos lacking the *Otpa* protein are devoid of dopaminergic neurons in the hypothalamus and the PT while overexpression of *Otp* can induce ectopic expression of dopaminergic markers, such as TH and dopamine transporter indicating that *Otp* can instruct dopaminergic identity (Ryu et al., 2007; Fernandes et al., 2013). *Otp* cooperates with another TF, *Sim1* (see below) to regulate the expression of TH, CRH, TRH, SST, OXT and AVP in the NPO and PT of zebrafish (Eaton et al., 2008; Borodovsky et al., 2009; Löhr et al., 2009; Fernandes et al., 2013).

As in the zebrafish, *Otp*^{-/-} mouse embryos lack diencephalic dopaminergic neurons of the diencephalospinal dopaminergic system (Acampora et al., 1999; Wang and Lufkin, 2000). Homozygous *Otp*^{-/-} mutant mice die soon after birth and display progressive impairment of crucial neuroendocrine developmental events such as reduced cell proliferation, abnormal cell migration and failure in terminal differentiation of neurons of the PVN, SON, and Arc (Acampora et al., 1999). Further analysis of *Otp* mutants revealed that *Otp* contributes to the patterning of the hypothalamus and preoptic region, and is required for differentiation of specific OXT, AVP, CRH and SST expressing cells (Acampora et al., 1999; Wang and Lufkin, 2000). Analysis of *Otp*^{-/-} mouse embryos demonstrated that *Otp*-expressing cells fail to properly migrate from the hypothalamus to the amygdaloid complex, leading to structural impairments in several amygdaloid nuclei (García-Moreno et al., 2010). This data suggest that in addition to its role as a developmental regulator of several neuroendocrine lineages, *Otp* is also an important regulator of migratory processes of other neuronal populations.

Sim1 and Arnt2–

Sim1 and *Arnt2* are two PAS (PER-Arnt-Sim) containing TFs belonging to the large basic loop-helix-loop (bHLH) family of TFs (Ema et al., 1996; Fan et al., 1996). Sim proteins present considerable sequence divergence from the *Drosophila melanogaster* protein. Sim form a heterodimeric protein complex with the aryl hydrocarbon receptor nuclear translocator (*Arnt*) to activate or repress their target genes containing the so called central midline enhancer (CME) or hypoxic response element (HRE) repeats (Moffett and Pelletier, 2000; Woods et al., 2008). Data from mice and zebrafish suggest that

the heterodimeric *Sim1*-*Arnt2* complex regulates hypothalamic differentiation *in vivo* (Michaud et al., 2000; Löhr et al., 2009).

Sim1 null and *Arnt2* null mice die shortly after birth and analysis of newborn brains indicates the lack of the hypothalamic aPV, PVN, and SON nuclei, phenocopying the *Otp* null phenotype (Michaud et al., 1998; Keith et al., 2001). However, *Otp* null mice show a dramatic decrease of almost 30% in brain size (Wang and Lufkin, 2000) while *Sim1*, *Sim2* and *Arnt2* null mice display developmental impairments that are correlated with deficits in neuronal migration and differentiation (Michaud et al., 1998, 2000; Goshu et al., 2004).

Sim1, *Arnt2* and *Otp* function along parallel pathways, as they are all required for *Sim2* expression in the PVN for the differentiation of the neurons secreting TRH, and in the aPV for the differentiation of the neurons that secrete SST. In the PVN and SON nuclei, these TFs are required for the maintenance of *Brn2* expression, a POU domain TF necessary for the development of OXT, AVP, and CRH producing neurons (Schonemann et al., 1995; Michaud et al., 1998, 2000; Acampora et al., 1999; Wang and Lufkin, 2000; Keith et al., 2001; Goshu et al., 2004).

Steroidogenic Factor 1 (SF-1)

The VMN of the hypothalamus is involved in the regulation of many homeostatic functions, such as the maintenance of energy balance, sexual behavior, anxiety and circadian rhythms (McClellan et al., 2006; Cheung et al., 2013). However, the development of the VMN is less characterized in comparison to the highly studied periventricular zone of the hypothalamus. The finding that SF-1 null mice lack a recognizable hypothalamic VMN, now allows the investigation of neuroanatomical and functional development of this non-peptidergic hypothalamic nucleus. SF-1 is an orphan nuclear receptor (also known as adrenal 4-binding protein; Ad4BP or Nr5a1). It was originally identified as a transcriptional regulator of cytochrome P450 steroid hydroxylases enzymes that are involved in the biosynthesis of steroid hormones (Omura and Morohashi, 1995; Parker and Schimmer, 1995). It is highly conserved both in structure and function throughout evolution (Luo et al., 1994; Achermann et al., 1999; Takase et al., 2000; Allen and Spradling, 2008). SF-1 is expressed in steroidogenic cells in the gonads, adrenal cortex and spleen as well as in the VMN and the anterior pituitary. At E11.0 SF-1 is expressed by diencephalic cells which will form the VMN (Ikeda et al., 2001; Tran et al., 2003). It is expressed as early as E9.0 during embryonic development of the mouse by cells which at later stages, will form the gonads in both sexes. At around E12.5, it shows sexual di-morphism with a higher expression in testes than ovaries (Luo et al., 1994; Ikeda et al., 2001; Sekido and Lovell-Badge, 2008). Notably, SF-1 is important for endocrine cell-fate specification (Lee et al., 2011). Mice lacking SF-1 expression die shortly after birth of adrenocortical insufficiency (Luo et al., 1994; Parker et al., 2002). These knock-out mice do not develop an adrenal gland or gonads. Similar defects in adrenal and gonadal development are also apparent in humans who carry mutations in the *sf-1* gene (Luo et al., 1994; Achermann et al., 1999). Disruption of SF-1

also results in structural and neuronal connectivity alterations in the VMN (Shinoda et al., 1995; Tran et al., 2003; Zhao et al., 2008; Cheung et al., 2013). Yet it seems that in the absence of functional SF-1 the initial migration and proliferation of the neuronal precursors remains unaffected, whereas it is important for terminal differentiation of these hypothalamic neurons (Tran et al., 2003). Interestingly, *in vitro* analysis of various promoters reveals potential SF-1 binding sites in the *fezf1*, *A2bp1*, *Nkx2-2*, *Slitrk1* and *Slitrk5* genes that are involved in neuronal differentiation and patterning (Kurrasch et al., 2007).

Receptors and Ligands Regulating Hypothalamic Development

The migration of a specific neuronal type into the hypothalamus, settlement of specific neuronal populations in distinct hypothalamic nuclei as well as their proper connectivity with a variety of target sites further requires extrinsic signals such as growth factors, neuropeptides and their receptors. These act concomitantly with the aforementioned intrinsic TFs to regulate the above processes. Moreover, several recent findings indicate that some neuropeptides, are involved in the development of neural circuits in which they function in the mature hypothalamus. This suggests that at least some neuropeptides act as “developmental autoregulators”. This section demonstrates the importance of these extrinsic factors for the patterning and assembly of neurocircuits in the developing hypothalamus.

Extrinsic Developmental Factors

Sonic hedgehog (SHH) which is probably the most characterized morphogen was shown to be crucial for the growth and axial patterning of the hypothalamus (Mathieu et al., 2002; Szabó et al., 2009; Alvarez-Bolado et al., 2012). Moreover, SHH was shown to directly regulate the expression of its cognate receptor Patched 1 (PTCH1) through which it probably signals to promote anterior-dorsal hypothalamic fate and to antagonize Nodal activity in the development of the posterior-ventral hypothalamus (Concordet et al., 1996; Koudijs et al., 2008; Szabó et al., 2009). SHH cooperates with Nodal in the maintenance of the anterior-dorsal hypothalamus (Mathieu et al., 2002) and with bone morphogenetic proteins (BMPs) to drive hypothalamic dopaminergic neuronal specification (Ohyama et al., 2005). Genetic disruption of BMP-receptor1a from *olig1* cell lineage led to a decreased number of dopaminergic and proopiomelanocortin (POMC) neurons and increased neuropeptide Y (NPY) neurons in the Arc leading to hypophagic phenotype (Peng et al., 2012). Although this genetic manipulation led to increased expression of the orexigenic AgRP, there was a profound impairment in their fiber numbers (Peng et al., 2012). As both Nodal and BMP are members of the transforming growth factor- β (TGF- β) superfamily, this data suggests interaction between SHH and TGF- β signaling pathways during hypothalamic development.

Wnt and its receptor Frizzled are also important regulators of hypothalamic differentiation and Wnt signaling components such as *wnt8b*, *Frizzled8a* and *Lef1* were shown to regulate the patterning, neurogenesis and differentiation of posterior hypothalamic cells of zebrafish (Kim et al., 2002; Lee et al., 2006; Russek-Blum et al., 2008). In addition, other members of the Wnt cascade were identified in the Arc of the mouse (Benzler et al., 2013). As the expression of SHH, Nodal, BMP and Wnt is maintained in the mature brain it would be interesting to see whether these molecules are released in a synaptic manner, or maintain their activity via a diffusion mechanism in the adult hypothalamus.

Peptides and Their Receptors

PAC1 (A.K.A ADCYAP1R1) which is the most specific (i.e., high-affinity) receptor for the pleiotropic neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) was shown to regulate the development of zebrafish dopaminergic and OXT neurons by controlling the rate of *Otp* protein synthesis (Blechman et al., 2007). The anorexigenic peptide Leptin is known to regulate metabolic related homeostatic functions in the hypothalamus through its cognate Leptin receptor (LepR; reviewed by Münzberg and Morrison, 2015). Interestingly, leptin was shown to directly regulate developmental neurite formation of Arc neurons (Bouret et al., 2004). It regulates the neural projections of both orexigenic NPY and anorexigenic POMC neurons (Bouret et al., 2012). This seemingly contradictory effect in which leptin regulates the plasticity of two neuronal populations with opposing effects on energy balance, could be partly explained by the developmental switch in leptin regulation of NPY neurons during the postnatal weaning maturational period (Baquero et al., 2014).

The roles of PACAP, Leptin and their receptors in regulating adult brain and hypothalamic functions were thoroughly investigated in the last decades (reviewed by Blechman and Levkowitz, 2013; Matsuda et al., 2013; Münzberg and Morrison, 2015). The above examples show that these peptidergic systems, which were previously considered as regulators of mature functions, also play a role in the developing hypothalamus. Thus, at least some neuropeptides regulate not only the function but also the development of the systems they control.

Adult Functions of Developmental Factors

Several key developmental factors have been found to be expressed in the mature hypothalamus, however, their post-developmental roles in homeostatic regulation remain elusive. This section presents the current data regarding the adult function of the developmental-related TFs described in previous sections of this manuscript.

Otp—

It has been suggested that a common ancestor of all ray-finned fish experienced a whole genome duplication event early in evolution, about 350 million years ago (Amores et al.,

1998; Vandepoole et al., 2004; Dehal and Boore, 2005; Brunet et al., 2006). As a result, the zebrafish genome contains two *Otp* genes, *otpa* and *otpb*, which share high sequence and expression-pattern homology and present partial redundancy in function (Ryu et al., 2007; Fernandes et al., 2013). *Otp* expression is maintained in the mature hypothalamus of mouse and zebrafish (Amir-Zilberstein et al., 2012; Herget et al., 2014; **Figure 3**). This suggests that alongside its crucial role in embryonic development, *Otp* is also involved in adult hypothalamic function. While *Otp*-null mice die shortly after birth and conditional allele for the gene currently does not exist, the duplication and partitioning of the gene in zebrafish allows both *otpa*^{-/-} or *otpb*^{-/-} fish to survive into adulthood (Ryu et al., 2007; Fernandes et al., 2013). This enables the investigation of *Otp*'s role in post-developmental and adult hypothalamic function. Data acquired from adult *Otp*-null zebrafish, point to the homeostatic activities *Otp* regulates in adult brains. Adult *Otpa*-null zebrafish demonstrated impaired anxiety-like behavior compared with their wild-type siblings in response to “novel-tank” diving test. Mutant fish spent more time in the top zone of the tank during the first 2 min of the assay suggesting that *Otp* is involved in the regulation of novelty related stressors (Amir-Zilberstein et al., 2012; Blechman and Levkowitz, 2013). *Otp* mutant zebrafish also display deficits in the molecular response of the hypothalamo-pituitary-adrenal (HPA) axis to stressors. These include the regulation of CRH transcription and cortisol response. Thus, *Otp* directly regulates CRH gene expression in zebrafish and mouse (Amir-Zilberstein et al., 2012). Moreover, *Otp* indirectly regulates the alternative splicing of the aforementioned

PAC1 receptor during stress adaptation phase that follows various homeostatic challenges (Amir-Zilberstein et al., 2012). Thus in addition to its role in hypothalamic development, *Otp* may act as a cellular sensor, which mediate between a given homeostatic challenge (“input”) and the following hypothalamic hormonal response (“output”). This assumption is reinforced by the finding that *Otp*-positive neurons in the zebrafish NPO modulate the visual motor response through the regulation of the melanopsin 4a (*opn4a*) receptor. This apparent *Otp*-mediated “non-visual” deep brain light-sensing system indicates that *Otp* neurons serve as an extra-ocular photoreception center in dark photokinesis behavior (Fernandes et al., 2012).

Taken together with its known role in regulating several types of neuropeptidergic neurons in the NPO/PVN, these findings suggest that *Otp* orchestrates the physiological response to environmental challenges. Given the importance of *Otp* in hypothalamic function, comprehensive research regarding its expression patterns and stress-induced molecular targets in response to physiological and psychological challenges, is further required.

Sim1–

Sim1 displays haploinsufficiency unveiling its function in metabolic regulation. *Sim1*^{+/-} mice possess a hypocellular PVN and are hyperphagic and obese with increased linear growth, hyperinsulinemia and hyperleptinemia, mainly under high fat diet conditions (Michaud et al., 2001; Holder et al., 2004; Kublaoui et al., 2006). *Sim1* heterozygotes display normal energy expenditure, and treatment with the melanocortin receptor

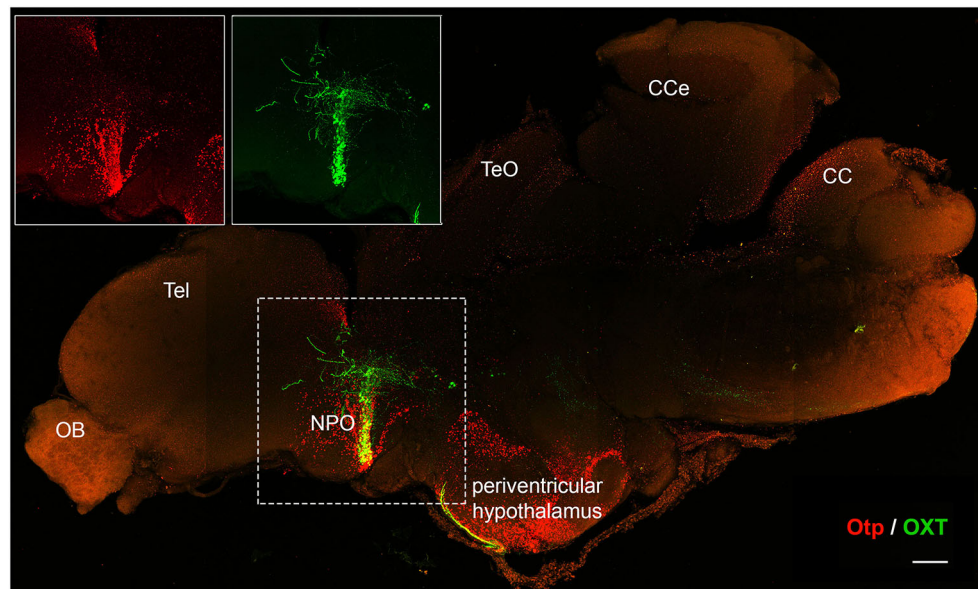


FIGURE 3 | *Otp* expression is maintained in the adult brain.

Immunofluorescence staining of *Otp* (red) and oxytocin (OXT) EGFP reporter (Blechman et al., 2011) (green) in a two year-old zebrafish brain. The image shows tiled maximum intensity projection of a mid-sagittal

section (150 μ m). Insets display separate single channel images of *Otp* and OXT in the NPO. CC, crista cerebellaris; CCe, corpus cerebelli; NPO, neurosecretory preoptic area; OB, olfactory bulb; Tel, telencephalon; TeO, tectum opticum. Scale bar, 200 μ m.

agonist, MTH, increases energy expenditure in both WT and *Sim1* heterozygous mice (Kublaoui et al., 2006). This phenotype is further supported by data from humans with balanced chromosomal translocations or genomic mutations, which interrupts the *Sim1* gene. In spite of their normal basal metabolic rate, these subjects usually suffer from early onset obesity, increased food intake, and display evidence of neurobehavioral abnormalities (Holder et al., 2000; Ramachandrapa et al., 2013).

Sim1^{+/-} mice display reduced expression of several hypothalamic neuropeptides such as TRH, CRH, AVP, and SST (Kublaoui et al., 2008). In line with their low OXT levels, *Sim1* heterozygotes also show higher sensitivity to the orexigenic effect of the OXT receptor antagonist, OVT (Kublaoui et al., 2008). Furthermore, intracerebroventricular administration of OXT to *Sim1*^{+/-} rescues the hyperphagic phenotype and reduces the characteristic weight increase of heterozygotes. Postnatal chemical ablation of *Sim1* expressing neurons leads to hyperphagic obesity and reduced expression of OXT and TRH. However, while *Sim1* heterozygotes or post-developmental knockouts display normal energy expenditure, ablation of *Sim1*-positive neurons leads to decreased energy expenditure (Xi et al., 2012). Postnatal PVN-specific ablation of *Sim1* combined with chow diet leads to a hyperphagic obesity phenotype. However, when fed with high-fat diet, the trend is reversed and these mice display reduced food intake and weight loss. Since PVN specific ablation of *Sim1* neurons also leads to increased *Sim1* expression in the Amygdala, the key regulating region of this phenotype remains to be determined (Xi et al., 2013).

In view of the above data, the authors suggested a model arguing that *Sim1* heterozygous phenotype of obesity and hyperphagia occur due to the *Sim1* regulatory effect on OXT which is also severely depleted in *Sim1* heterozygotes and melanocortin receptor-4 (*Mc4r*), both of which are known to function in appetite regulation (Kublaoui et al., 2006, 2008; Tolson et al., 2010). The accumulating data further support the fact that at least some of the phenotypes correlated with lack of *Sim1* are caused by the perturbation of its mature brain functioning rather than from developmental impairments.

SF-1—

SF-1 positive neurons are directly involved in the regulation of body weight in the mature brain. This function is mediated, among others, by their responsiveness to the hormone leptin. Hence, specific deletion of leptin receptor in SF-1 neurons results in defects in the ability to maintain normal energy homeostasis and these mice display increased body weight (Dhillon et al., 2006). In addition, there are some indications that SF-1 may directly regulate the expression of the brain-derived neurotrophic factor (BDNF; Tran et al., 2003, 2006), a growth factor which is involved in energy balance (Xu et al., 2003). Yet, it is not clear whether SF-1 indeed regulates BDNF *in vivo*, and if so—what the physiological significance of this regulation is (Dhillon et al., 2006).

Alongside its role in the maintenance of energy balance SF-1 may be involved in the modulation of the HPA axis

in response to stress. SF-1 heterozygous mice develop normal VMN and pituitary, but have defects in their adrenal and in their stress response. These mice display abnormal circulating levels of ACTH and corticosterone during the day and following stressful challenges (Bland et al., 2000). In addition, mice with CNS-specific SF-1 knock-out display anxiety-like behaviors in response to various environmental challenges (Zhao et al., 2008). Beyond the developmental defects that disrupt the normal stress response, *in vitro* experiments indicate the involvement of SF-1 in the direct regulation of the CRH receptor-2 gene, and in SF-1 knock-out mice there is a marked reduction in CRH receptor expression in the VMN (Zhao et al., 2008).

Concluding Remarks

The hypothalamus regulates brain and body functions by controlling the activity of a variety of neuropeptide-containing cell types. By doing so, it allows vertebrates to orchestrate multiple homeostatic processes in order to adapt to the ever-changing environment, thereby maintaining the organism's survival and reproduction. Therefore, mechanistic understanding of the patterning and differentiation of the hypothalamus may further shed light on the function of the mature hypothalamus.

The increased use of zebrafish as a model organism allows the combination of powerful genetic tools with high-resolution imaging techniques to advance our knowledge regarding the molecular pathways governing hypothalamic development. One obvious advantage in utilizing both mammalian and non-mammalian models is the ability to gain wider knowledge on evolutionarily conserved regulatory pathways. However, a prerequisite for practical use of the accumulating scientific knowledge is to understand the neuroanatomical organization of the hypothalamus in the key models.

Although the knowledge regarding nucleus-specific markers and TFs continue to expand, many hypothalamic nuclei remain “uncharted”. Thus, the identification of new regulators of hypothalamic development and function is of fundamental importance. In this regards, the introduction of innovative genome editing methodologies, such as the transcription activator-like effector nuclease (TALEN) or the clustered regularly interspaced short palindromic repeats (CRISPR) genome editing methods now allow efficient genetic manipulation and analysis of new candidate genes (Doudna and Charpentier, 2014; Wright et al., 2014).

Recent findings have demonstrated that some genetic pathways that are involved in hypothalamic development, also play a role in mature hypothalamic functions (summarized in **Table 1**). Thus, key developmental factors maintain their expression in the mature hypothalamus. As developmental perturbation of these genes might lead to lethality, the use of conditional and inducible knockout models is necessary. For example, *Sim1* post-developmental knockouts demonstrate the valuable scientific knowledge that can be achieved when using such models, unveiling the highly important role of

TABLE 1 | Key functions demonstrated for transcription factors expressed in both developing and mature hypothalamus.

Factor	Developmental function	Function in adult
Otp	<ul style="list-style-type: none"> Differentiation of the neurons of the aPV, PVN, and SON (Blechman et al., 2007; Eaton et al., 2008) Development of diencephalic dopaminergic neurons in zebrafish and mouse (Ryu et al., 2007) Required for expression of TH, CRH, TRH, SST, OT and AVP in the dorsal preoptic area and posterior tuberculum of zebrafish (Eaton et al., 2008; Löhner et al., 2009; Fernandes et al., 2013) 	<ul style="list-style-type: none"> Regulation of CRH expression (Amir-Zilberstein et al., 2012) Maintenance of <i>opn4a</i> in the aPO, thus regulating dark photokinesis (Fernandes et al., 2012)
Sim1	<ul style="list-style-type: none"> Differentiation of TRH neurons in the PVN and SST neurons in the aPV (Schonemann et al., 1995; Michaud et al., 1998, 2000; Acampora et al., 1999; Wang and Lufkin, 2000; Keith et al., 2001; Goshu et al., 2004); Maintenance of <i>Brn2</i> expression in the PVN and SON nuclei (Schonemann et al., 1995; Michaud et al., 1998, 2000; Acampora et al., 1999; Wang and Lufkin, 2000; Keith et al., 2001; Goshu et al., 2004) Together with <i>Otp</i>, required for expression of TH, CRH, TRH, SST, OT and AVP in the dorsal preoptic area and posterior tuberculum of zebrafish (Eaton et al., 2008; Löhner et al., 2009; Fernandes et al., 2013) 	<ul style="list-style-type: none"> Control of OXT expression to affect appetite regulation (Kublaoui et al., 2006, 2008; Tolson et al., 2010)
SF-1	<ul style="list-style-type: none"> Terminal differentiation of VMN neurons (Tran et al., 2003) 	<ul style="list-style-type: none"> Involvement in energy balance via maintenance of leptin-receptor (Dhillon et al., 2006) Regulation of BDNF expression (Xu et al., 2003) Modulation of the peripheral HPA axis in response to stress (Bland et al., 2000) Direct regulation of <i>crhr2</i> expression <i>in vitro</i> (Zhao et al., 2008)

Sim1 in feeding and metabolic regulation in the mature hypothalamus.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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