Cell and Tissue Research (2019) 375:5–22
https://doi.org/10.1007/s00441-018-2900-4

REVIEW

Anatomy, development, and plasticity of the neurosecretory hypothalamus in zebrafish

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Received: 10 May 2018 / Accepted: 20 July 2018 / Published online: 14 August 2018

Abstract

The paraventricular nucleus (PVN) of the hypothalamus harbors diverse neurosecretory cells with critical physiological roles for the homeostasis. Decades of research in rodents have provided a large amount of information on the anatomy, development, and function of this important hypothalamic nucleus. However, since the hypothalamus lies deep within the brain in mammals and is difficult to access, many questions regarding development and plasticity of this nucleus still remain. In particular, how different environmental conditions, including stress exposure, shape the development of this important nucleus has been difficult to address in animals that develop in utero. To address these open questions, the transparent larval zebrafish with its rapid external development and excellent genetic toolbox offers exciting opportunities. In this review, we summarize recent information on the anatomy and development of the neurosecretory preoptic area (NPO), which represents a similar structure to the mammalian PVN in zebrafish. We will then review recent studies on the development of different cell types in the neurosecretory hypothalamus both in mouse and in fish. Lastly, we discuss stress-induced plasticity of the PVN mainly discussing the data obtained in rodents, but pointing out tools and approaches available in zebrafish for future studies. This review serves as a primer for the currently available information relevant for studying the development and plasticity of this important brain region using zebrafish.

Keywords Hypothalamus · Paraventricular nucleus · Zebrafish · Stress

Introduction

The hypothalamus is an evolutionarily ancient part of the brain, critical for the survival and maintenance of basic life functions. It functions as the master homeostatic regulator for a wide range of physiological processes including stress response, food intake, thermoregulation, fluid balance, circadian rhythm, growth and reproduction, as well as cognitive and social behaviors (Saper and Lowell 2014). The hypothalamus acts as an integrating node, where it receives diverse sensory inputs coming from the local neuronal afferents as well as peripheral circulatory systems. These inputs are continuously evaluated against the ideal basic "set points" for parameters such as hormone and metabolite levels, body temperature, and salt concentration to restore optimal physiology or homeostasis (Saper and Lowell 2014; Burbridge et al. 2016). Moreover, in the face of an ever changing and challenging environment, the hypothalamus initiates adaptive processes via hormonal and neural outputs to restore homeostasis, called allostasis (McEwen and Wingfield 2003; Juster et al. 2010), which is pivotal for the survival of the organism and the species.

The hypothalamus is composed of functional cell types conserved from the brains of annelids to vertebrates including zebrafish, rodents, and humans (Tessmar-Raible et al. 2007; Machluf et al. 2011; Löhr and Hammerschmidt 2011; Herget et al. 2014; Saper and Lowell 2014; Xie and Dorsky 2017). There is a remarkable similarity in the general anatomy and organization of the hypothalamus in vertebrates. As opposed to other regions of the central nervous system like the cortex, spinal cord, or cerebellum that have a columnar architecture, the hypothalamus comprises a plethora of distinct nuclei as

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well as less anatomically defined areas that are organized in a three-dimensional patchwork fashion interacting with each other and with the regions outside of the hypothalamus to regulate physiology and behavior. The hypothalamus can be anatomically divided into four divisions from rostral to caudal: preoptic, anterior, tuberal, and mammillary hypothalamus, each of which has a lateral, medial, and (medial-most) periventricular zone (Markakis 2002; Szarek et al. 2010; Burbridge et al. 2016). The preoptic area contains key integrative circuitry for thermoregulation, electrolyte balance, and reproduction. The anterior hypothalamus, comprising the supraoptic nucleus (SON), paraventricular nucleus (PVN), suprachiasmatic nucleus (SCN), and anterior periventricular nucleus (aPV), regulates stress response, circadian rhythms, feeding, and other homeostatic processes. The tuberal hypothalamus comprises the arcuate nucleus (ARC), median eminence (ME), lateral hypothalamic area (LHA), ventromedial (VMH), and dorsomedial (DMH) hypothalamus. The pituitary stalk (infundibulum) emerges from the ventral surface of this central region of the hypothalamus. The tuberal hypothalamus possesses integrative circuitry for feeding and output circuitry for sexual behavior, aggression, and various autonomic and endocrine responses. The mammillary hypothalamus, which includes the mammillary bodies, plays a crucial role in arousal and stress response, as well as spatial and episodic memory through its output to the hippocampus (Béracochéa 2005; Vann and Nelson 2015).

Vital for the diverse functions of the hypothalamus are the neurosecretory cells that differ morphologically, connect to different targets, and produce different neuropeptides or neurohormones, which serve various critical functions. These neurosecretory cells can be divided into two groups: the magnocellular neurosecretory cells and the parvocellular neurosecretory cells. The magnocellular neurons located in the PVN and SON project their axons directly to the posterior lobe of the pituitary gland (neurohypophysis), where they release oxytocin (OXT) and arginine-vasopressin (AVP) into the blood circulation (Swanson and Sawchenko 1983; Landgraf and Neumann 2004). In contrast to the magnocellular population, the parvocellular neurons located in the PVN and other periventricular nuclei like ARC, aPV, and DMH, project to the median eminence and release the neurohormones, which reach the anterior pituitary (adenohypophysis) via the hypophyseal portal system. One such neurohormone released from the parvocellular neurons in PVN is the corticotropin-releasing hormone (CRH), also known as the primary stress hormone. In response to a stressor, CRH promotes the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary (Vale et al. 1983), which in turn causes the release of the final effector of the stress response-glucocorticoids from the adrenal cortex, constituting the hypothalamus-pituitary-adrenal (HPA) stress response axis (Charmandari et al. 2005). Thus, PVN, located at the apex of HPA axis, is a major center for the regulation of stress response whose dysfunction can be seen in variety of stress-linked disease states (Herman et al. 2016; Herman and Tasker 2016).

The PVN is arguably one of the best-characterized and most well-studied nuclei in the hypothalamus. However, although the anatomy and development of the PVN have been studied primarily using rodents as a model for a long time (Swanson 1980; Swanson and Sawchenko 1980; Sawchenko and Swanson 1983; Swanson and Sawchenko 1983; Rho and Swanson 1989; Simmons and Swanson 2008), still many questions remain concerning how the PVN develops, and little is known about how its development is influenced by the environment. For example, although much information is available on the stress-induced plasticity of the PVN in adult rodents, much less is known about the effect of stress on the PVN during development. It is inherently difficult to examine environmentally induced plasticity during development in animals that develop in utero since precise regulation of environmental parameters is challenging. The zebrafish, *Danio rerio*, has recently emerged as a powerful genetic model for hypothalamic development in vertebrates (Machluf et al. 2011) and offers a particularly attractive model to study how the environment affects development. The zebrafish is a highly attractive vertebrate model to study neurodevelopment, circuit function, and plasticity owing to a number of features: optically transparent embryos that develop rapidly and externally, cheap housing costs, short generation time and large progeny size, and a highly advanced and versatile toolkit for genetic, pharmacological, optogenetic, and behavioral manipulations (Grunwald and Eisen 2002; Arrenberg and Driever 2013; Portugues et al. 2013; Holtzman et al. 2016; Li et al. 2016; Albadri et al. 2017; Orger and de Polavieja 2017). Belonging to the vertebrate subphylum, fish and mammals share the general overall organization, basic structures, and functions of major hypothalamic regions and nuclei. Comparative studies between zebrafish and rodents have revealed conserved molecules dictating the specification and differentiation of hypothalamic neurons as well as shared neuropeptides executing the diverse hypothalamic functions. In this review, we will discuss the anatomy, development, and plasticity of the neurosecretory hypothalamus, primarily focusing on the PVN. Specifically, we will describe in detail studies that address the anatomy and cell type composition of the putative homologous region to the mammalian PVN in fish. Next, we will review studies on the development of different cell types in the neurosecretory hypothalamus in both mouse and in fish. Lastly, we will discuss rodent data on stress-induced plasticity of the neurosecretory hypothalamus focusing on the PVN. In the outlook, we then discuss how zebrafish can be used to study the environmentally driven plasticity of the neurosecretory hypothalamus.

Anatomy of the neurosecretory hypothalamus in zebrafish

The PVN is a key neurosecretory nucleus of the anterior hypothalamus, which is vital for the functioning of several homeostatic processes. The functional cell types of this nucleus are conserved from fish to humans. Neurons in this nucleus produce secretory peptides, which are transported through axons towards the pituitary. Two peptides, arginine vasopressin (AVP) and oxytocin (OXT), are transported to the neurohypophysis, the neuronal posterior part of the pituitary, and released directly into the general body circulation. Other peptides produced in different cells act indirectly in that they are transported to the median eminence and released into the portal system of the adenohypophysis, the glandular anterior part of the pituitary. There, they induce or inhibit the release of hormones into the general bloodstream. These inhibiting or releasing peptides include corticotropin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH), methionine enkephalin (mENK), leucine enkephalin (lENK), neurotensin (NTS), cholecystokinin (CCK), vasoactive intestinal peptide (VIP), and somatostatin (SST) in rats (Swanson and Sawchenko 1983; Mezey et al. 1985; Simmons and Swanson 2009). Peripheral release of OXT is crucial for parturition and lactation, while AVP is essential for controlling diuresis and blood pressure. Further, mammalian OXT-producing neurons are also activated by stress, food intake and social attachment (Strand 1999; Onaka et al. 2012). CRH is the primary stress hormone released by the brain, triggering the release of ACTH in the adenohypophysis (Vale et al. 1983). NTS reduces blood pressure, and NTS+ cells cluster densely within the PVN (Carraway and Leeman 1973). Enkephalins are opiates, and serve analgesia, while CCK and VIP regulate gastrointestinal function, and VIP also triggers vasodilation (Strand 1999). SST inhibits the release of growth hormone in the adenohypophysis, and SST-producing cells reside in a different region than that occupied by AVP+/OXT+ cells (Swanson and Sawchenko 1983). In fish, Avp also regulates water homeostasis (Amer and Brown 1995) and jointly with Oxt controls blood pressure (Chan 1977). The functional importance of the PVN is however not restricted to peptide synthesis. Since the release of their peptides needs to be constantly adjusted to current demands imposed by the status of the animal and its environment, these cells also integrate information coming from various sensory systems to adapt their hormonal output (Ferguson et al. 2008). The system formed by the hypothalamus and the pituitary is therefore a central interface in which neuronal, hormonal, and vascular systems are connected.

The zebrafish-neurosecretory preoptic area (NPO) as the putative homolog of the PVN

While the anatomy of the rodent PVN was well described in decades of work, information about the homologous nucleus in fish is far less extensive. The putative homolog of the PVN in adult teleosts has been located within the preoptic area (PO) and is traditionally referred to as the neurosecretory preoptic area or preoptic nucleus (NPO). However, the PO is thought to not be a part of the hypothalamus, as it is located between the anterior and postoptic commissures and therefore rostral to the hypothalamus. The PVN in mammals is not located in the mammalian region also called preoptic area but in the alar hypothalamus (Nieuwenhuys et al. 2008).

Pioneering studies of the NPO in the 1960s found neurosecretory somata forming dense projection bundles connecting to the pituitary, the hypothalamo-neurohypophysial tracts, in several fish species, such as the lantern shark, common minnow, and the walking catfish (Braak 1962; Sathyanesan 1969; Bhargava 1969). The actinopterygian PO was subdivided into several nuclei based on histology (Braford and Northcutt 1983; Wullimann et al. 1996; Rupp and Northcutt 1998), including the anterior parvocellular, the magnocellular, the posterior parvocellular, and the suprachiasmatic nuclei. Homology between the magnocellular preoptic nucleus in adult zebrafish (Wullimann et al. 1996) and the PVN has been suggested in a report on agouti-related protein (AgRP) and α-melanocyte-stimulating hormone (MSH) (Forlano and Cone 2007). While some information has thus been obtained in adult fish, the anatomical situation in larvae was unclear. Due to the homogeneous appearance of gray matter in the larval zebrafish hypothalamus, cytoarchitectonic boundaries of hypothalamic nuclei cannot be identified. However, the molecular neuroanatomy of the hypothalamus can be defined using a field homology approach (Puelles and Medina 2002) by analyzing the expression of conserved regionalized transcription factors (Puelles and Rubenstein 2003; Domínguez et al. 2015) and area-specific neuropeptides to identify similar regions in zebrafish larvae. Therefore, the identification of the larval NPO was performed by studying expression domains of transcription factors defining the developing PVN in other vertebrates together with the expression of relevant neurosecretory peptides in the larval zebrafish brain (Herget et al. 2014; Herget and Ryu 2015).

Transcription factor expression delineating the PVN-homologous region in vertebrates

Generally in tetrapods, both the paraventricular and supraoptic nuclei are formed within the supraopto-paraventricular region (SPV), the most anterodorsal part of the hypothalamus, which is bordered by the prethalamus/eminentia thalami posteriorly, and the telencephalon dorsally (Puelles and Rubenstein 2003; Osório et al. 2010; Morales-Delgado et al. 2011; Moreno and González 2011; Moreno et al. 2012; Puelles et al. 2012; Domínguez et al. 2013). The SPV in mammals is considered to be the neuroendocrine part of the developing hypothalamus

(Morales-Delgado et al. 2011). This region is defined by the expression of the transcription factor *Orthopedia* (*Otp*) and surrounded by regions expressing *Distalless* (*Dlx*) (Simeone et al. 1994; Wang and Lufkin 2000; Shimogori et al. 2010). *Dlx5* flanks the *Otp* domain in the developing mouse brain, since it is expressed in surrounding structures of the subpallium, prethalamus, hypothalamus, and the preoptic region. The *Otp*+ cells of the PVN are generally excluded from bordering *Dlx*+ domains in tetrapods (Puelles and Rubenstein 2003; Bardet et al. 2008; Morales-Delgado et al. 2011). It appears that the SPV in any given tetrapod can be identified by the expression of *Otp* and the absence of *Dlx*. The SPV is similarly bordered by *Islet-1* (*Isl1*) expression in turtles and anurans (Moreno et al. 2012; Domínguez et al. 2013). In addition, the bordering prethalamus and eminentia thalami express *Aristaless* (*Arx*) (Miura et al. 1997). The topology of *Otp* expression is conserved in tetrapods and likely a plesiomorphic feature of all vertebrates (Bardet et al. 2008). Therefore, the expression of *Arx*, *Dlx*, and *Isl1* can be used as markers to identify bordering regions surrounding the *Otp*-expressing SPV, and these markers were used to determine the location and extent of the teleostean NPO based on evolutionary conservation (Herget et al. 2014). In zebrafish, *Otp* exists as two paralogs, *otpa* and *otpb* (Del Giacco et al. 2006; Blechman et al. 2007; Ryu et al. 2007). The transcription factor *isl1* was found to be expressed in regions bordering *otpa* medially, rostrally, caudally, and dorsally. *dlx5a* also borders *otpa* medially, rostrally, caudally (in the PO), and dorsally (in the prethalamus, PTh). Lastly, *arx*, which is another marker for the PTh, also forms a neighboring expression domain dorsal to *otpa*. The arrangement of regions and borders of *isl1*, *dlx5a*, *arx*, *otpa*, and *otpb* is summarized in a schematic illustration (Fig. 1). In summary, the conserved expression of transcription factors in the PO of larval zebrafish allowed the identification of the SPV, which contains the NPO.

Cell types of the PVN/NPO

Not only the spatial arrangement of transcription factor expression but also the biochemical identity and many functions of neuropeptides are conserved between the SPV in mammals and the teleostean NPO. Conserved expression of neuropeptides within the previously delineated *otpa*-positive PO would provide a strong argument in support of the proposed homology between the larval zebrafish NPO and the SPV. Expression patterns of typical mammalian PVN neuropeptides were previously described in the zebrafish, including *avp*, *oxt*, *sst1.1*, *vip*, and *crh* (Unger and Glasgow 2003; Chandrasekar et al. 2007; Eaton et al. 2008; Löhr et al. 2009; Machluf et al. 2011; Wolf and Ryu 2013). Our work (Herget et al. 2014) then extended the analysis of typical peptidergic cell types found in the mammalian PVN. Cells were found expressing *cck*, *nts*, *oxt*, *penka*, *penkb*, *vip*, *avp*, *sst1.1*, or *crh* within the volume defined by *otpa* expression. Since distinct clustering of neuropeptidergic cell types is documented in rodents, the spatial distribution of cell types was then analyzed to determine the chemoarchitecture of the NPO. All analyzed cell types, except *trh*, form clusters within the dorsal half of the *otpa*-positive part of the PO (Fig. 2). This comprehensive 3D map of neuropeptides showed a small group of cells producing *cck* at the rostral border of the NPO and cells producing *avp*, *oxt*, *crh*, *penka*, *nts*, or *sst1.1* as dense and intermingled clusters. In contrast, cells producing *penkb* or *vip* appeared to reside in separate subregions of the NPO. Both *avp*+ and *oxt*+ cells, typical markers of the PVN, cluster within the *otpb*-expressing subregion of the larger *otpa* domain. While in adult fish, *trh* expression was localized in PPa and PM (Diaz et al. 2002), and the cluster of *trh*+ cells in 5 dpf larvae was found at the rostroventral end of the preoptic area, away from the *avp*+ and *oxt*+ clusters (Löhr et al. 2009). Based on these results, a rostrocaudal arrangement of cell types can be approximated: *penkb*, *cck*, *avp*, *sst1.1*, *crh*, *nts*, *penka*, *oxt*, *vip*, and *penkb*.

A common phenomenon in the mammalian PVN is that cells do not only express one of the neurosecretory peptides but also express two or even more at the same time. For example, AVP and mENK were found to colocalize in rat neurohypophyseal terminals (Martin and Voigt 1981). OXT-producing cells can also express CRH, ENK, or CCK (Martin and Voigt 1981; Vanderhaeghen et al. 1981; Rossier et al. 1983; Swanson and Sawchenko 1983; Levin and Sawchenko 1993). The stress hormone CRH can be coexpressed with AVP, OXT, ENK, NTS, CCK, and/or VIP in rats (Burlet et al. 1983; Hökfelt et al. 1983; Roth et al. 1983; Sawchenko et al. 1984; Mezey et al. 1985; Piekut and Joseph 1986; Swanson et al. 1986; Sawchenko 1987; Whitnall and Gainer 1988; Ceccatelli et al. 1989; Swanson and Simmons 1989; Arima et al. 2001; Dabrowska et al. 2011). The classification of NPO cell types should therefore take into account not only the expression of solely one neuropeptide.

The 3D chemoarchitectural map of the neuropeptide expression was further extended to include the degrees of coexpression of two neuropeptides in the same cell by performing systematic pairwise comparisons (Herget and Ryu 2015). This is relevant as the coexpression properties of PVN cells are subject to stress-induced plasticity, with different types of stress influencing the expression levels of different neuropeptides in mammals (Swanson et al. 1986; Harbuz and Lightman 1989; Swanson 1991). The larval zebrafish offers an attractive system to dissect the mechanistic basis of such environment-induced plasticity in the hypothalamus. In the coexpression analysis (Herget and Ryu 2015), many of the peptides produced by densely intermingled cells of the larval zebrafish NPO were found not to be coexpressed, while some

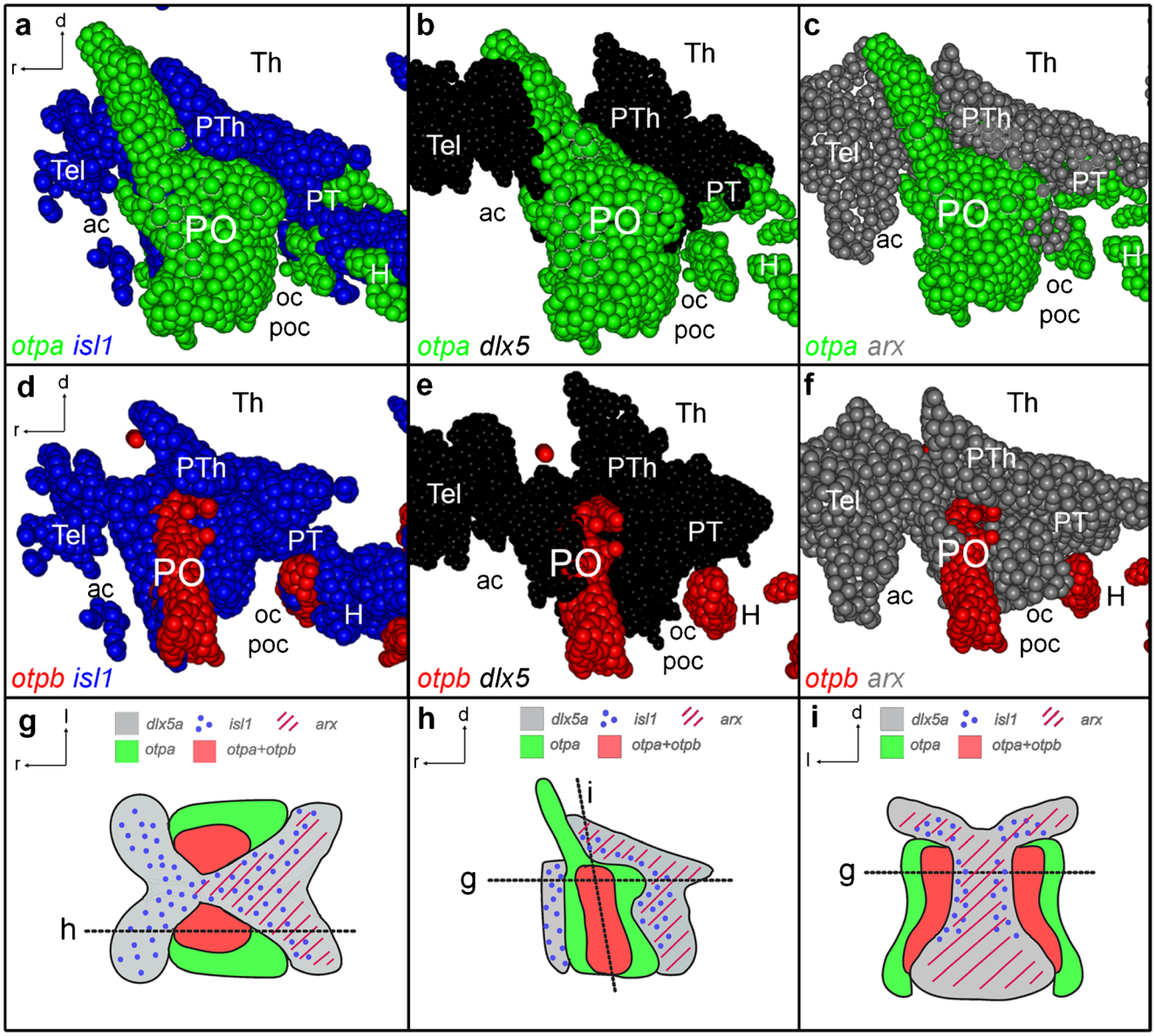


Fig. 1 Schematic overview of transcription factor arrangement in and around the PO at 5 days post fertilization (dpf). **a-f** Schematic sagittal views of cell clusters expressing *otpa* in comparison to *isl1* (a), *dlx5* (b), or *arx* (c) illustrate their spatial relations. The smaller *otpb*-expressing cluster is part of the *otpa*-expressing cluster, and therefore also fits into gaps formed by *isl1* (d), *dlx5* (e), and *arx* (f). **g-i**: Schematic horizontal (g), sagittal (h), and frontal (i) planar sections illustrate borders of *isl1*, *dlx5a*, and *arx* expression in relation to that of *otpa* and *otpb* (Modified from Herget et al. 2014). For abbreviations, see list

neuropeptide combinations show occasional, low or moderate levels of coexpression. However, high degrees of coexpression for certain neuropeptide combinations such as *avp* + *crh* and *cck* + *penkb* were observed. The high degree of coexpression of *avp* and *crh* observed has immediate functional implications. Similar to CRH, AVP stimulates ACTH secretion (Gillies and Lowry 1979; Rivier and Vale 1983). In humans, AVP/CRH coexpression increases with age, and a connection with stress has been implied (Raadsheer et al. 1993). Either *penka* or *penkb* was found to be coexpressed in both *crh*-positive and *avp*-positive cells. The concerted action of coreleased CRH and ENK is proposed to fine-tune stress regulation (Pretel and Piekut 1990). For mammals subjected to osmotic stress, ENK and CRH levels are increased, but restraint and swimming stress only elevated CRH, not ENK (Harbuz and Lightman 1989). Such findings highlight the functional implications of studying coexpression differences. Furthermore, biochemical switching in response to changes in environmental conditions has been suggested as a relevant biological mechanism in PVN neurons (Kiss 1988; Swanson 1991). Dynamic adaptation of neuroendocrine transcription to changes in supply or demand for neuropeptides has also been suggested in zebrafish larvae (Kurrasch et al. 2009).

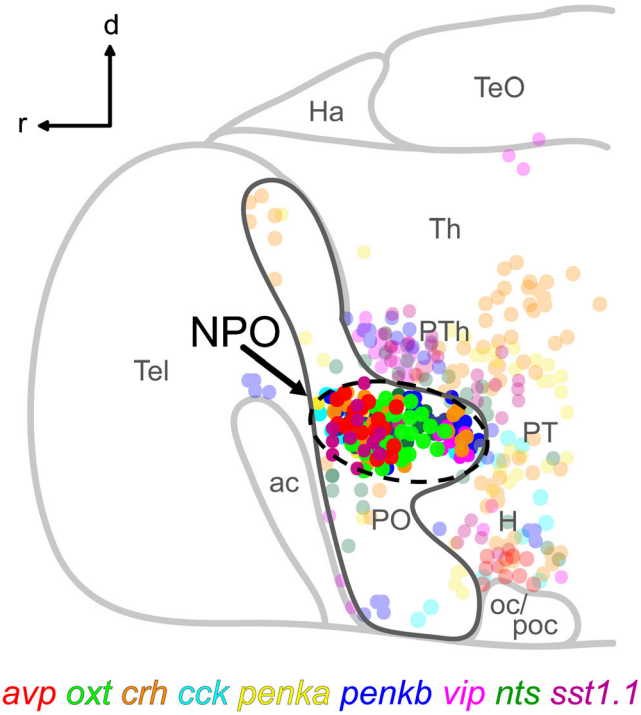


Fig. 2 The chemoarchitecture of the neurosecretory preoptic area. Representative layout of the lateral view of a 5 dpf larval zebrafish brain showing the location of the NPO (dashed line) within the *otpa*-positive part (dark gray line) of the preoptic area and the spatial distribution of nine cell types expressing the indicated neuropeptides. For abbreviations, see list. (Adapted from Herget and Ryu 2015)

Projections of NPO neurons in zebrafish

Since long-range projections of PVN neurons are lost during the sectioning steps typically performed in rodent brains, such projections are difficult to analyze. The larval zebrafish brain can be easily studied without sectioning, allowing the visualization and hodological tracing of long-range fibers throughout the entire brain. The projections of NPO neurons form dense and complex bundles that innervate the pituitary but also reach far into the spinal cord (Herget et al., unpublished observation). Even the projections of the small cluster of Oxt-positive cells reach the pituitary and the spinal cord in the form of complex entangled fibers, and innervation of other brain regions can also be observed. This dense network of fibers originating from specific NPO cell types can be dissected by single-cell reconstruction. To visualize individual neurons of the NPO in their entirety, we utilized the Brainbow technique, which labels individual cells with different stochastically expressed fluorescent proteins due to Cre-mediated recombination, thereby visualizing them with different colors (Livet et al. 2007; Lichtman et al. 2008). This approach could visualize individual cells in the dense cluster of NPO cells and their projections (e.g., to the pituitary) after heat shock inducible Cre recombination. The utility of Brainbow in zebrafish is limited by the availability of specific promoters. Using a comprehensive screening of cis-regulatory regions of the transcription factor *otp*, our lab had previously isolated an evolutionarily conserved vertebrate enhancer module (called *otp*ECR6), which allows targeted expression in NPO (Gutierrez-Triana et al. 2014). Combining NPO-specific expression with the Brainbow technique using this transgenic approach, innervation targets of different NPO cells can be visualized, as illustrated by differently colored cells projecting to different neurohypophyseal subregions (Fig. 3). This method combined with immunohistological staining for specific neuropeptides can yield cell-type specific projectomes of the neurons in the NPO area. For example, the projectome analysis of Oxt cells using the Brainbow technique revealed the existence of two main types of Oxt cells in the larval zebrafish brain: those that innervate the pituitary and those that innervate diverse brain regions (Herget et al. 2017). Similar to the situation in the adult rat and the adult midshipman fish, but in contrast to the situation in the adult trout, these two cell types are mutually exclusive and can be distinguished based on morphological and anatomical criteria. (Herget et al. 2017)

Development of the neurosecretory hypothalamus

A hypothalamic primordium is induced during neural plate formation, in the medial region of the prosencephalon for all vertebrates (Woo and Fraser 1995; Kobayashi et al. 2002). Induction of the hypothalamus is mediated by signaling from morphogens secreted by surrounding tissues that create a positional map in the neural plate. As the hypothalamus is induced and patterned, a set of transcription factors then drive the specification of individual hypothalamic nuclei. For a detailed account of hypothalamic induction, patterning, and neurogenesis, we refer to a recent review (Xie and Dorsky 2017). Later, the specification of distinct hypothalamic brain nuclei and neuronal populations relies on transcription factors and other cofactors of transcriptional machinery. Insights into these networks have been obtained by analyzing phenotypes and gene expression patterns in mutant mouse and zebrafish embryos and by targeted knockdown of candidate hypothalamic specification genes. Among several transcription factors involved in hypothalamus development, three transcription factors play major roles in the neuronal specification of the PVN in rodents and NPO in zebrafish, namely *otp*, *sim1*, and *fezf2*. Specific roles of these transcriptional regulators are discussed below. For further discussion on the role of these transcription factors on hypothalamic development and function, readers are referred to Machluf et al. (2011) and Biran et al. (2015). For a broad overview of the role of Otp, Sim1, and Fezf2 on the development of neuroendocrine

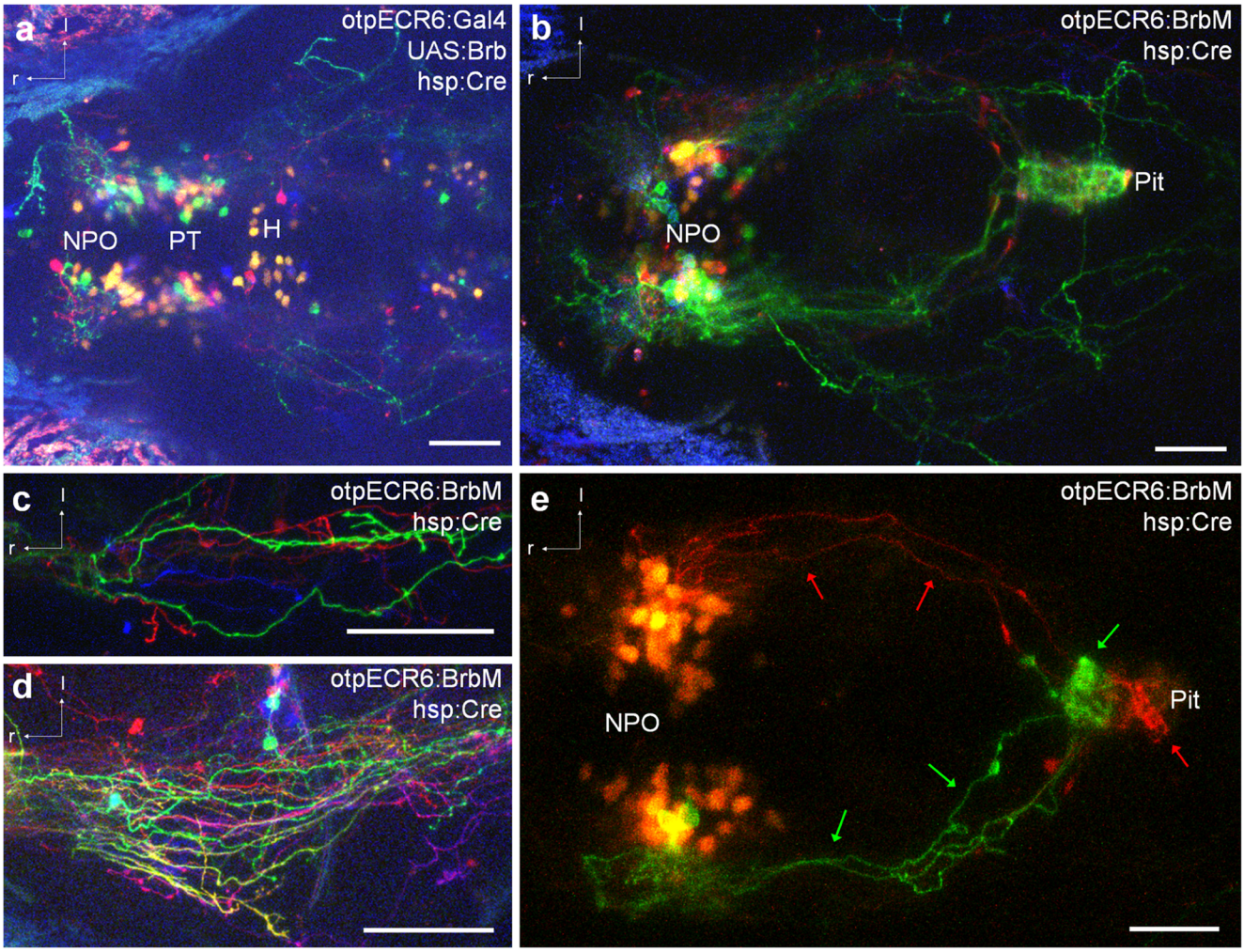


Fig. 3 Brainbow under the control of *otp*ECR6. **a** The Gal4/UAS system using *otp*ECR6:Gal4 and UAS:BrainbowM is not entirely specific for the NPO, since expression also appears in clusters of the PT and H. **b** Linking *otp*ECR6 directly to BrainbowM gives NPO cells different colors after Cre recombination. **c** Fibers in a fish with one integration of BrainbowM. **d** Fibers in a fish with multiple integrations of BrainbowM. **e** The different colors resulting from recombination clarify the different projections and target regions (arrows) of NPO cells towards the pituitary, which would be unrecognizable with only one color (Modified from Herget et al. 2017). For abbreviations, see list. Scale bars 50 μm

neurons in the mammalian hypothalamus, readers are referred to an excellent recent review by Alvarez-Bolado (2018).

The homeodomain transcription factor Orthopedia (Otp) and the bHLH-PAS transcription factors Sim1/Arnt2

The homeodomain-containing transcription factor OTP is highly conserved across species (Simeone et al. 1994; Lin et al. 1999; Umesono et al. 1999). Single minded-1 (SIM1) and aryl hydrocarbon receptor nuclear translocator-2 (ARNT2) belong to the large superfamily of basic loop-helix-loop Per-Arnt-Sim (bHLH-PAS) transcription factors. OTP, together with the heterodimeric SIM1/ARNT2 complex, is required for the development of virtually all magno- and parvocellular neurons of PVN and SON, as well as the neurons in the aPV (Michaud et al. 1998; Michaud et al. 2000; Keith et al. 2001). The OTP null, SIM1 null, and ARNT2 null mouse embryos lack AVP, OXT, CRH, and TRH neurons in the PVN, SON, and aPV, while OTP null mice also lack SST neurons in the ARC (Michaud et al. 1998; Acampora et al. 1999; Wang and Lufkin 2000; Caqueret et al. 2005; Szarek et al. 2010). These defects are linked with reduced proliferation, abnormal cell migration, and impaired differentiation of neuroendocrine progenitors. Also, OTP null mice lack the diencephalic dopaminergic neurons of the A11 group located near the central periaqueductal gray (Ryu et al. 2007). Furthermore, OTP null and SIM1 null mice show reduced cell proliferation resulting in hypocellular PVN and SON (Wang and Lufkin 2000). Also,

in OTP null mouse embryos, the *Otp*-expressing cells do not migrate properly from the hypothalamus to the amygdaloidal complex, resulting in structural impairments in several nuclei of the amygdala (García-Moreno et al. 2010).

Reflecting their parallel mode of action, *Otp* and *Sim1* show an identical expression pattern within the hypothalamus and the amygdala (Wang and Lufkin 2000). However, in contrast to OTP, SIM1/ARNT2 act during the late differentiation stage of the postmitotic hypothalamic progenitors, while OTP is also required at early stages to regulate proliferation and specification of neuronal precursor cells (Michaud et al. 1998; Acampora et al. 1999). Notably, SIM1 heterozygous mice become hyperphagic and obese, indicating a gene dosage effect of SIM1 (Duplan et al. 2009; Xi et al. 2012). These mice have fewer numbers of AVP-, OXT-, and TRH-positive neurons, while there was no effect on CRH and SST neurons. Downstream of OTP and SIM1/ARNT2, a POU domain containing transcription factor, BRN2, and a close homolog of SIM1, SIM2, regulate the development of PVN, SON, and a part of aPV neurons. Both *Brn2* and *Sim2* expressions were observed to be reduced in OTP and SIM1 null mutant mice. BRN2 mutant mice do not show defects in the initial hypothalamic development and migration of progenitors, but rather in the maturation of CRH, AVP, and OXT neurosecretory neurons (Nakai et al. 1995; Schonemann et al. 1995). SIM2 mutant mice show reduced numbers of SST and TRH neurons in the PVN and aPV. SIM1, acting upstream of SIM2, can partially compensate for the loss of SIM2. In addition, ARNT2 can also function as a heterodimerization partner of SIM2 (Goshu et al. 2004).

Remarkably, the transcriptional machinery regulating hypothalamic specification is highly conserved during evolution. In zebrafish, Otp and Sim1/Arnt2 are required for the development of Oxt, Avp, Crh, Trh, and Sst neurons in the NPO (Eaton and Glasgow 2006; Blechman et al. 2007; Eaton and Glasgow 2007; Eaton et al. 2008; Löhr et al. 2009; Fernandes et al. 2013; Wolf and Ryu, unpublished data). The Otp promoter harbors several evolutionarily conserved noncoding sequences (ECR) and *otp*ECR6 expression was specifically found to overlap with the central core region of the NPO, where *crh*, *avp*, *oxt*, and *penka* -positive cells are clustered (Gutierrez-Triana et al. 2014). Additionally, *brn2* expression in the NPO is downregulated in zebrafish embryos lacking either Sim1/Arnt2 or Otp function (Löhr et al. 2009). Interestingly, Otp and Sim1 were first implicated in the development of diencephalic dopaminergic neurons in studies performed on zebrafish (Blechman et al. 2007; Ryu et al. 2007; Borodovsky et al. 2009; Löhr et al. 2009). The zebrafish was also the first model system where upstream regulators of Otp and Sim1 activity were found. The bHLH protein Olig2 activates *sim1a* expression in a specific progenitor pool critical for the formation of diencephalic dopaminergic neurons (Borodovsky et al. 2009). *otp* expression in the NPO is regulated by Nodal, FGF8, and SHH while Nodal but not FGF8 and SHH regulates *otp* expression in the posterior tuberculum (PT) (Del Giacco et al. 2006). Further, the homeobox gene *prox1*, a homolog of drosophila *prospero*, acts upstream of *otp* to specify dopaminergic neurons in the PT (Pistocchi et al. 2008). Two other factors acting upstream of *otp* are the zinc finger-containing transcription factor Fezf2 and the G-protein coupled receptor Pac1. Fezf2 (discussed below) acts as a transcriptional activator for *otp*. Pac1 is a high affinity receptor for pituitary adenylate cyclase-activating polypeptide (PACAP) which was shown to regulate *otp* on the posttranscriptional level to specify dopaminergic and serotonergic neurons in the PT and Oxt neurons in the NPO (Blechman et al. 2007).

The zinc finger protein Fezf2

The forebrain embryonic zinc finger-like protein Fezf2 (also known as Fezl) is one of the first markers delineating the prospective forebrain and plays a critical role in establishing regional subdivisions within the diencephalon and in the development of the telencephalon and hypothalamus (Jeong et al. 2007). A hypomorphic mutant allele in zebrafish (*tof*^{m808}) disrupting the gene encoding for Fezf2 displays a strong reduction in the number of hypothalamic Oxt, serotonergic, and dopaminergic neurons (Guo et al. 1999; Levkowitz et al. 2003; Rink and Guo 2004) where Fezf2 controls monoaminergic neuron development in a noncell-autonomous fashion. The loss of monoaminergic neurons is maintained through adulthood, while the development of Oxt neurons in the NPO is merely delayed (Blechman et al. 2007). In addition *fezf2* mutant zebrafish have defects in telencephalic glutamatergic population (Allalou et al. 2017). Fezf2 is also important for early development of the posterior hypothalamus in zebrafish (Wolf and Ryu 2013). Coordinated activities of Fezf2, Otp, Sim1a, and Foxb1.2 were found to be crucial for the establishment of the mammillary area subdomains and the specification of *vip*- and *urotensin1* (*uts1*)-positive neurons (Wolf and Ryu 2013).

In mouse, *Fezf2* is expressed in the thalamic eminence, the prethalamus, the hypothalamus, and the dorsal telencephalon, which later becomes confined to the deep layers of the cortex (Shimizu and Hibi 2009). Similar to zebrafish, *Fezf2* in mouse controls the rostro-caudal patterning of the diencephalon (Jeong et al. 2007). Additionally, it represses the caudal diencephalic fate and establishes the prethalamic fate (Hirata et al. 2006; Shimizu and Hibi 2009) and is also expressed in the developing mouse hypothalamus (Hirata et al. 2004). It is also required for the specification and development of subcerebral projection neurons in the cortex and their axonal projection to the spinal cord and brainstem (Chen et al. 2005a; Molyneaux et al. 2005; Chen et al. 2005b; Shimizu and Hibi 2009).

The expression of *fezf2* is under tight regulation of the canonical Wnt signaling pathway (Hashimoto et al. 2000; Matsuo-Takasaki et al. 2000; Yang et al. 2001; Jeong et al.

2007). *fezf2* expression was found to be upregulated following the over-expression of Wnt antagonists, and Wnt8b/Fz8a ligand receptor interaction downregulates the transcription of *fezf2*, which is required for dopaminergic neuron development in the forebrain (Hashimoto et al. 2000; Russek-Blum et al. 2008). This indicates that *fezf2* expression is repressed by canonical Wnt signaling and is crucial for determining the dopaminergic neuron population size during early forebrain development. In addition, Fezf2 controls the expression of proneural gene *neurogenin 1*, which is necessary for zebrafish dopaminergic neuron development in PT (Jeong et al. 2006).

Otp has been found to be a downstream effector of Fezf2. Otp null fish display the same dopaminergic neuron phenotype as *fezf2* (*tof*^{m808}) deficiency (Blechman et al. 2007). Different Otp protein levels were observed to regulate specific differentiation programs that resulted in distinct dopaminergic and oxytocinergic identities. Fezf2 also regulates Lhx5 in zebrafish and a targeted knockdown of *lhx5* resulted in deficits in both Oxt and dopaminergic neurons (Machluf et al. 2011). Furthermore, two more downstream targets of Fezf2 have also been identified: transcription factor (TF) eomesa or Tbr2 and the Lim homeobox protein Lhx2 (Chen et al. 2011).

Stress-induced alteration of the neurosecretory hypothalamus

To cope with a stressful situation, the body initiates a complex repertoire of physiological and behavioral adaptive processes that bring back stability (Chrousos 1998). This fundamental process of maintaining stability through change, allostasis, is critical for organisms to actively adjust to predictable and unpredictable events (McEwen 2000; McEwen and Wingfield 2003). The allostatic response is mounted by the neuroendocrine stress response system comprising of the HPA axis both in fish and in mammals (McEwen 2007; Alsop and Vijayan 2009; Denver 2009; Joëls and Baram 2009). Among several hypothalamic nuclei that are directly involved in regulating HPA axis response to stressors, the PVN stands out as a principal integrator of stress signals. The PVN houses three distinct populations of neurons: dorsomedial and anterior parvocellular neurons that project to the median eminence and directly regulate the HPA axis; magnocellular neurons in the anterior, medial and posterior magnocellular cell groups which secrete AVP and OXT; and brainstem and spinal cord projecting neurons located in the lateral, dorsal, and ventromedial parvocellular division that regulate autonomic functions (Swanson 1980; Swanson and Sawchenko 1983). For the acute stressed condition in rodents, dorsomedial parvocellular neurons upregulate FOS, NGFI-B, CREB, and MAPK expression immediately after exposure to stressors (Kovacs and Sawchenko 1996; Khan and Watts 2004). At a later time point after stressor exposure, *Crh* and *Avp* transcripts are increased (Herman et al. 1992; Herman et al. 1995). In the case of several chronic stress paradigms in rodents like chronic unpredictable stress, social subordination, and social defeat, dorsomedial parvocellular neurons expressed elevated mRNA levels of *Crh, Avp*, and *GluR5* subunit of kainite-preferring glutamate receptor (Imaki et al. 1991; Herman et al. 1995; Makino et al. 1995; Albeck et al. 1997; Ziegler et al. 2005; Keeney et al. 2006) but decreased *GR*, beta 1 and 2 subunit of *GABA-A* receptor, and *NMDA-R2B* receptor subunit (Herman et al. 1995; Makino et al. 1995; Cullinan and Wolfe 2000). In mammals, stress-induced plasticity is also exhibited in the form of variation in the coexpression of neuropeptides in PVN cells, with different types of stress influencing the expression levels of different neuropeptides (Swanson et al. 1986; Harbuz and Lightman 1989; Swanson 1991). For example, *Crh* mRNA in the PVN was found to be increased in response to hypertonic saline, restraint, and swim stress but not to cold stress (Harbuz and Lightman 1989). Different chronic levels of circulating corticosterone in rats appeared to change the ratio of CRH and AVP, but not neurotensin (NT), released from the axon terminals of neurons in the PVN (Swanson 1991). Furthermore, the number of CRH neurons in the PVN varies with the circulating levels of adrenal steroid hormones in the body (Swanson et al. 1986). These plasticity mechanisms illustrate the role of biochemical switching of information flow in anatomically fixed circuits.

Using various stress paradigms in zebrafish as well as mouse, Otp was found to modulate the expression of Crh as well as the splicing factor ataxin 2-binding protein-1 (A2BP1/Rbfox-1) (Amir-Zilberstein et al. 2012). Also, the G protein coupled receptor PAC1, which is a known A2BP1/Rbfox-1 splicing target and an important mediator of Crh activity, was observed to be alternatively spliced in response to a stressor exposure. The generation of PAC1-hop mRNA isoform by alternative splicing was found to be essential for termination of *crh* transcription, normal activation of the HPA axis, and adaptive anxiety-like behavior. In a further study to explore the role of Otp in hypothalamic development and function in zebrafish (Wircer et al. 2017), it was found that developmental mutations in the zebrafish paralogous gene *otpa* but not *otpb* affect both stress response and social preference. These behavioral phenotypes were linked to developmental alterations in a subset of Oxt neurons that coexpress Crh and project mostly to the hindbrain and spinal cord. Ablation of this neuronal subset specifically reduced adult social preference behavior without affecting stress response, thereby revealing the contribution of this specific Oxt cluster to social behavior and differentiating it from the general *otpa*−/− deficits. Another transcription factor, the Wnt/ß-catenin effector Lef1 was described to be required for the differentiation of anxiolytic hypothalamic neurons in zebrafish and mice (Xie et al. 2017).

CRH-synthesizing parvocellular neuroendocrine cells (PNCs) of the PVN are innervated by glutamatergic and GABAergic axons that, along with monoaminergic and peptidergic inputs, are crucial for regulating HPA axis output (Ulrich-Lai and Herman 2009). In fact, the proportion of GABAergic synapses in the PVN is significantly higher than any other brain region, giving it a substantial inhibitory tone (Cole and Sawchenko 2002; Miklos and Kovacs 2002; Park et al. 2007). Some of the GABAergic innervation to the PVN comes from neurons in the peri-PVN region (Roland and Sawchenko 1993), which in turn is targeted by several limbic brain regions, thereby allowing limbic modulation of the HPA axis (Herman et al. 2002). Microinjection of glutamate into the PVN has been found to stimulate HPA axis output, whereas ionotropic glutamate receptor antagonist administration before exposure to stress blunts the neuroendocrine stress response (Darlington et al. 1989; Cole and Sawchenko 2002; Ziegler et al. 2005). In contrast to the glutamatergic transmission, GABAergic synaptic transmission resets the baseline HPA axis activity. For instance, microinjections of GABA-A receptor antagonists in the absence of a stressor strongly activate PVN neurons and the HPA axis (Cole and Sawchenko 2002), indicating the release from the tonic inhibitory GABA tone (a phenomenon known as disinhibition).

A single acute stress event is sufficient to affect signaling at glutamatergic synapses on parvocellular neuroendocrine cells (PNCs). The exposure to stress results in an increase in the ratio of AMPA receptor to NMDA receptor-mediated transmission (Kuzmiski et al. 2010). This is due to a long-lasting decrease in NMDA receptor signaling that results from the downregulation of postsynaptic NMDARs by local release of CRH during stress (Kuzmiski et al. 2010). Preinjection of the NMDAR antagonists MK-801 or memantine led to an increase in *Fos* and *Crh* mRNA expression in the PVN, and elevated ACTH and corticosterone levels in the blood, following exposure to two homotypic stressors (Lee et al. 1994; Givalois et al. 2000; Pechnick et al. 2006; Armario et al. 2008). This suggests that NMDA receptor downregulation plays an important role in amplifying responses to subsequent stressors. Exposure to chronic stress has also been reported to cause long-term changes in HPA-axis activity and responsiveness, resulting in baseline hyperactivity and hypersensitivity to a novel stressor (Akana et al. 1992a; Akana et al. 1992b; Armario et al. 2004). The synaptic plasticity at GABAergic synapses on PNCs is different from the classical metaplasticity in that the timing of the induction after the initial exposure to stress is of utmost importance. In naive (not exposed to stress) animals, GABAergic synapses on PNCs do not exhibit long-term plasticity (Cusulin et al. 2013; Inoue et al. 2013). However, after exposure to either a physical or a psychological stressor, both long-term potentiation (LTP) (Inoue et al. 2013) and long-term depression (LTD) (Cusulin et al. 2013) can be induced at GABAergic synapses. Chronic or repeated stress can also induce several changes in PNC GABAergic synapses. These may include alterations in the expression of GABA-A receptor subunits (Cullinan and Wolfe 2000; Verkuyl et al. 2004) and a reduction in retrograde endocannabinoid signaling (Wamsteeker et al. 2010). Chronic stress can also lead to changes in GABAergic synaptogenesis and subcellular synapse redistribution. Under nonstress conditions, GABAergic synapses are uniformly distributed between somatic and dendritic compartments of PNCs (Miklos and Kovacs 2012). Chronic stress causes a dramatic increase in the total number of GABAergic synaptic contacts, and there is an alteration in relative distribution of these contacts, such that dendritic inputs become more highly represented (Miklos and Kovacs 2012).

Although much has been described about the mechanisms of plasticity (Bains et al. 2015; Herman and Tasker 2016), there has been a dearth of information about the activity of PNCs and CRH-producing neurons in vivo, particularly in response to stress exposure. Recently, we used two-photon calcium imaging on intact larval zebrafish to record the activity of Crh cells to determine how these neurons respond to different stressor intensities in an intact animal (Fig. Fig. 4) (Vom Berg-Maurer et al. 2016). By combining behavioral and physiological measures, we first determined how acute changes in environmental conditions lead to different levels of stress axis activation. Then, changes in the frequency and amplitude of Ca^{2+} transients in individual Crh neurons were measured in response to such stressors. The response magnitude of individual Crh neurons was found to covary with stressor intensity. Furthermore, stressors led to the recruitment of previously inactive Crh neurons in an intensity-dependent manner, thus increasing the pool of responsive Crh cells. Also, stressor-induced activity was observed to be highly synchronized among Crh neurons, and also across hemispheres, which ensures that the overall output of the HPA axis matches the severity of the threat.

Outlook: analysis of stress-induced NPO plasticity in zebrafish

To dissect both acute and chronic stress-induced plasticity of NPO neurons, precise control of stressor delivery is critical. To achieve this, we have first identified the specific promoters that allow targeting cells of the HPI axis in zebrafish. These include both the promoter targeting the NPO cells (*otp*ECR6, mentioned above) and the promoter of steroidogenic acute regulatory protein (*star*), which allows targeting the steroidogenic cells within the interrenal gland (Gutierrez-Triana et al. 2015). The specific promoter for targeting the corticotroph cells of the anterior pituitary is already identified and available (Liu et al. 2003). Combining some of these promoters with either optogenetic or genetic ablation tools, we have then successfully achieved means to precisely regulate the activity of

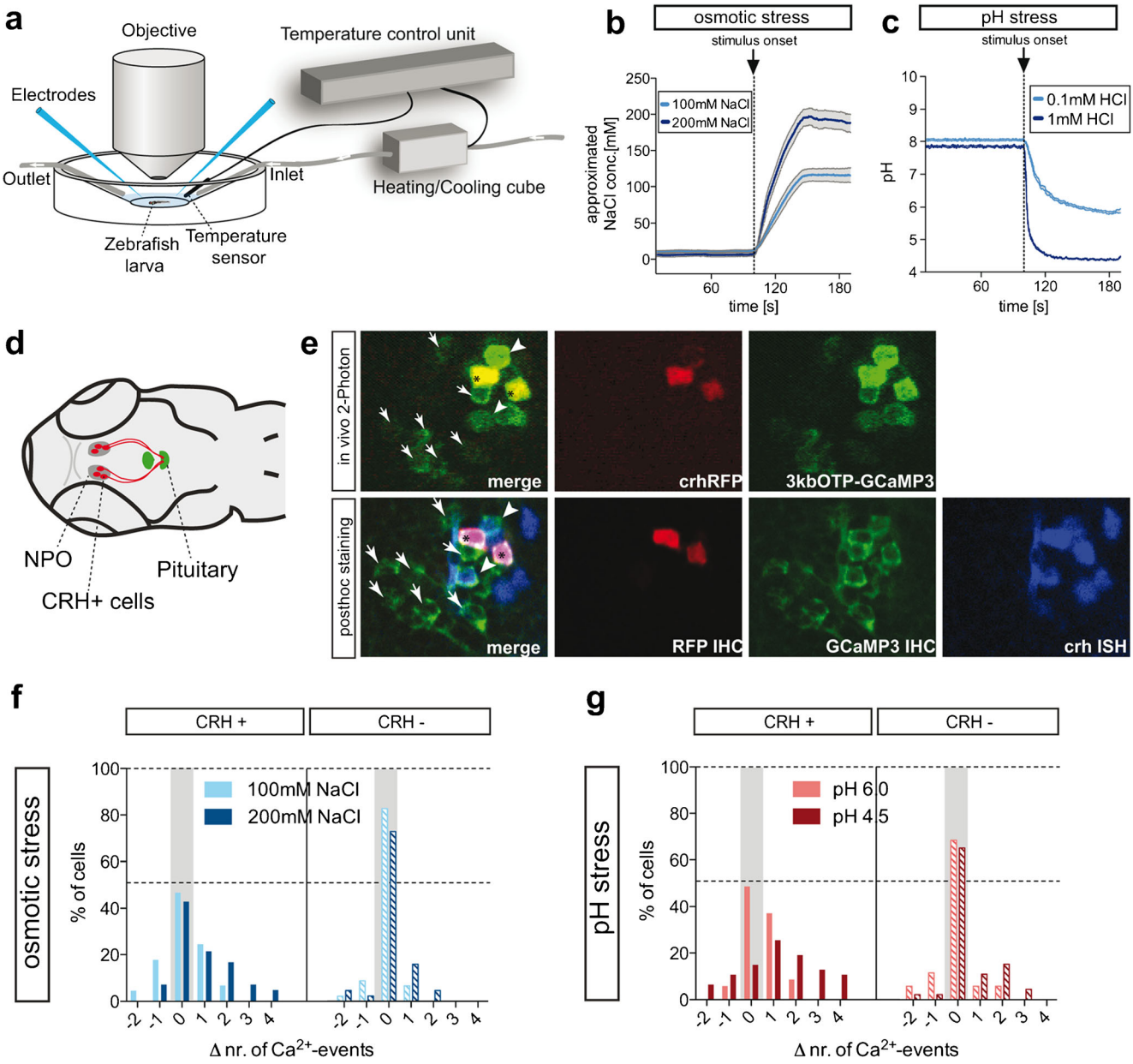
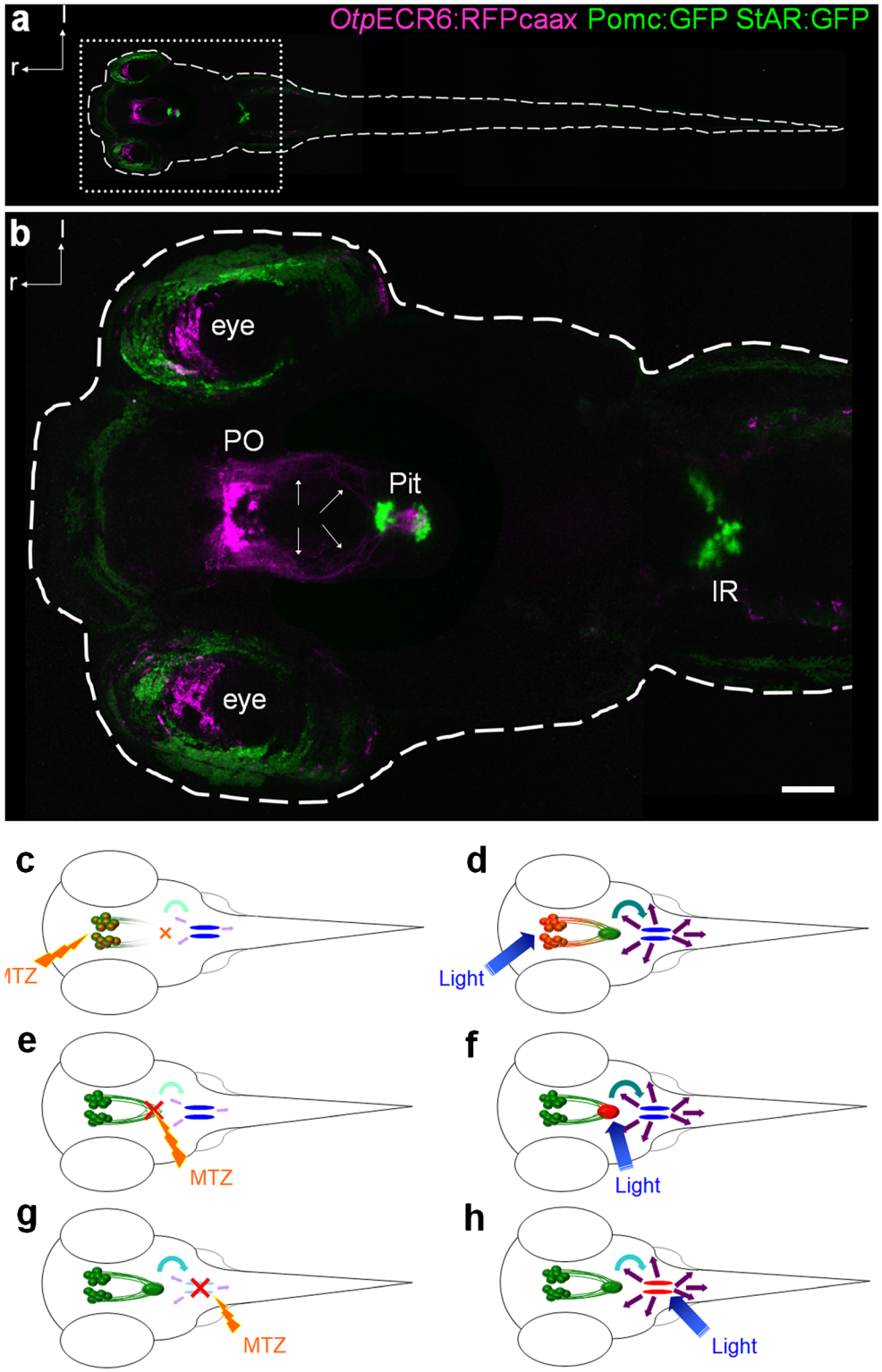


Fig. 4 Two-photon Ca^{2+} imaging of stress-induced activity changes in CRH-positive cells in the NPO. **a** A cartoon of the imaging chamber. **b** The time course of NaCl concentration changes can be precisely measured in the imaging chamber by measuring changes in the liquid junction potential. **c** Average traces of $n = 9$ experiments (HCl_{high}) and $n = 14$ experiments (HCl_{low}). **d** A cartoon of a larval head where CRH+ cells are indicated in red and the NPO area in dark gray. **e** Red-labeled CRH cells (marked with asterisk) in the transgenic lines that express RFP in CRH cells and GCaMP3.0 in the NPO region were identified in vivo (top). The same fish was stained post hoc for RFP (red), GCamP (green) and CRH (blue). **f**, **g** Frequency distribution histograms show that as stressor intensity increased, the number of nonresponsive CRH cells decreased, and the magnitude of change in the number of Ca^{2+} events increased. In contrast, most CRH negative cells remained nonresponse, regardless of stressor intensity. Shaded in gray are nonresponsive cells (active and inactive) that did not show a change in the number of Ca^{2+} events in response to the stimulus (Modified from Vom Berg-Maurer et al. 2016)

corticotroph cells or steroidogenic cells using light or chemical treatment to result in altered endocrine and behavioral stress response (De Marco et al. 2013; Gutierrez-Triana et al. 2015; De Marco et al. 2016) (Fig. Fig. 5). In an ongoing study, we are currently using transgenic zebrafish lines that express the optogenetic tool *Beggiatoa* photoactivatable adenylyl cyclase (bPAC) with targeted expression in the interrenal gland in order to alter endocrine output of the HPA axis during development. Our results indicate that using such transgenic zebrafish lines, we can create animals that have been exposed to elevated levels of cortisol during development. Interestingly, these animals exhibit stable alteration in the stress response and molecular signatures in the NPO, indicating that exposure to elevated levels of cortisol during



development can program the NPO in the larval zebrafish. Further elucidation of molecular, cellular, and physiological alterations as a function of elevated levels of cortisol during a defined developmental time window will then allow us to gain systematic and comprehensive information on the effect of stress on developing NPO.

In short, the transgenic access to the HPA axis available now in zebrafish combined with the detailed neuroanatomical, developmental, and physiological information that we have at hand on the NPO neurons offers a unique opportunity to mechanistically dissect the effect of acute and chronic stress on NPO plasticity.

◀ **Fig. 5** The stress axis is now fully accessible and can be manipulated in larval zebrafish. **a** Dorsal in vivo view of a triple transgenic larva at 6 dpf expressing markers for the NPO and its projections (*otp*ECR6:RFPcaax), the pituitary (Pomc:GFP), and the interrenal gland (StAR:GFP). **b** Magnified view of the head (boxed in **a**) showing the three elements of the stress axis. The animal shown was digitally skinned dorsally to remove autofluorescent skin signal, but autofluorescence in the eyes remain. Spinal projections were digitally removed to show only the fibers of the hypothalamohypophyseal tract (arrows). **c–h** Functional manipulation of stress axis organs is now possible, as illustrated in schematic drawings showing the hypothalamohypophyseal complex (green) and the interrenal glands (blue). The transgenic access established by our lab can be used to ablate NPO cells using MTZ in *Tg(otpECR6-E1b:nfsb-GFP)* larvae (**c**), which removes the innervation of the pituitary (cross), thereby reducing ACTH output (light green curved arrow), thereby reducing cortisol output (light purple arrows). NPO cells can also be activated using optogenetic proteins (**d**), which under light activation increases the release of ACTH (dark green curved arrow), thereby increasing the release of cortisol (dark purple arrows). The pituitary can be ablated (**e**), reducing ACTH output (light green curved arrow), thereby reducing cortisol output (light purple arrows), or optogenetically activated using photoactivatable adenylyl cyclase (**f**), increasing the release of ACTH (dark green curved arrow), thereby increasing the release of cortisol (dark purple arrows). The interrenal cells can also be ablated (**g**), reducing cortisol output (light purple arrows), or optogenetically activated using photoactivatable adenylyl cyclase (**h**), increasing cortisol output (dark purple arrows). For abbreviations, see list. Scale bar 100 μm. Modified from Herget (2015)

Funding This study was funded by the Max Planck Society, the University Medical Center of the Johannes Gutenberg University Mainz, the German Federal Office for Education and Research (Bundesministerium für Bildung und Forschung) grant number 01GQ1404 to S.R., and German Research Foundation (Deutsche Forschungsgemeinschaft) SPP1926 Next Generation Optogenetics grant to S.R.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Abbreviations ac , anterior commissure; ACTH, adrenocorticotropic hormone; AgRP , agouti-related protein; AMPA , α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid–glutamate receptor agonist; aPV, anterior periventricular nucleus; ARC, arcuate nucleus; ARNT2, aryl hydrocarbon receptor nuclear translocator-2; Arx , aristaless-related homeodomain transcription factor; AVP , arginine vasopressin; bHLH, basic helix loop helix; CCK , cholecystokinin; CRH , corticotropin-releasing hormone; d, dorsal; Dlx, distal less homeodomain transcription factor; DMH, dorsomedial hypothalamus; ECR, evolutionarily conserved region in the enhancer; ENK, enkephalin; Fezf2, forebrain embryonic zinc finger-like protein; H, hypothalamus; Ha, habenula; HPA, hypothalamo-pituitary-adrenal axis; IR, interrenal gland; Isl-1, islet-1 homeodomain transcription factor; lENK, leucine enkephalin; LHA, lateral hypothalamic area; MA, mammillary area; mENK, Methionine enkephalin; MSH, Melanocyte-stimulating hormone; MTZ, Metronidazole (drug for nitroreductase cell ablation system); NMDA, *N*-methyl-D-aspartate–glutamate receptor agonist; NPO, neurosecretory preoptic area/preoptic nucleus; NTS, neurotensin; oc, optic chiasm; Otp, Orthopedia homeodomain transcription factor; OXT, oxytocin; penka, proenkephalin a; penkb, proenkephalin b; Pit, pituitary; PM, magnocellular preoptic nucleus; PNC, parvocellular neuroendocrine cells; PO, preoptic area; poc, postoptic commissure; pomc, proopiomelanocortin; PPa, anterior parvocellular preoptic nucleus; PPp, posterior parvocellular preoptic nucleus; PT, posterior tuberculum; PTh, prethalamus; PVN, paraventricular nucleus; r, rostral; SIM1, single minded-1; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; SPV, supraoptoparaventricular region; SST/sst1., 1 somatostatin; STAR, steroidogenic acute regulatory protein; Tel, telencephalon; TeO, optic tectum; Th, thalamus; TRH, thyrotropin-releasing hormone; VIP, vasoactive intestinal peptide; VMH, ventromedial hypothalamus

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