The aversive brain system of teleosts: Implications for neuroscience and biological psychiatry

Rhayra Xavier do Carmo Silva[1,2], Monica Gomes Lima-Maximino[3], Caio Maximino[1]

[1] Laboratório de Neurociências e Comportamento "Frederico Guilherme Graeff", Instituto de Estudos em Saúde e Biológicas, Universidade Federal do Sul e Sudeste do Pará

[2] Programa de Pós-Graduação em Neurociências e Biologia Celular, Instituto de Ciências Biológicas, Universidade Federal do Pará

[3] Laboratório de Neurofarmacologia e Biofísica, Departamento de Morfologia e Ciências Fisiológicas, Universidade do Estado do Pará – Campus VIII/Marabá

Abstract

Defensive behavior is a function of specific survival circuits, the "aversive brain system", that are thought to be conserved across vertebrates, and involve threat detection and the organization of defensive responses to reduce or eliminate threat. In mammals, these circuits involve amygdalar and hypothalamic subnuclei and midbrain circuits. The increased interest in teleost fishes as model organisms in neuroscience created a demand to understand which brain circuits are involved in defensive behavior. Telencephalic and habenular circuits represent a "forebrain circuit" for threat processing and organization of responses, being important to mounting appropriate coping responses. Specific hypothalamic circuits organize neuroendocrine and neurovegetative outputs, but are the less well-studied in fish. A "midbrain circuit" is represented by projections to interneurons in the optic tectum which mediate fast escape responses via projections to the central gray and/or the brainstem escape network. Threatening stimuli (especially visual stimuli) can bypass the "high road" and directly activate this system, initiating escape responses. Increased attention to these circuits in an evolutionary framework is still needed.

Keywords: Fear; Anxiety; Aversive brain system; Comparative neuroanatomy; Teleost fish

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1. Introduction

Defensive behavior is a function of specific survival circuits that are highly conserved across vertebrates (LeDoux, 2012), and involve threat detection, as well as the organization of species-specific defensive responses to reduce or eliminate threat (LeDoux, 2012). In mammals, these circuits involve sensory systems that detect conditioned and unconditioned threatening stimuli, amygdalar circuits that select appropriate behavioral and neuroendocrine responses and promote aversive learning, hypothalamic circuits for both defensive behavior and neuroendocrine responses, and midbrain circuits that control the output (LeDoux, 2012). This survival circuit for defensive behavior links this appraisal phase, in which the global organismic state is determined based on threat detection, to a response phase, in which "the instrumental physiological and behavioural responses are determined and executed" (Andersen et al., 2016, p. 5). Different threat levels restrict attention "in the current global organismic state [to make] the organism focus on a short-term motive to […] survive a threat" (Andersen et al., 2016, p. 6).

In fish, defensive circuits have not been thoroughly described, but there is an increasing awareness that defensive behavior is a fundamental function in these animals (Andersen et al., 2016; Kalueff et al., 2012; Kittilsen, 2013). As a result, fish are increasingly being used in neurobehavioral research (Gerlai, 2014; Hall et al., 2014; Kalueff et al., 2014b; Stewart et al., 2015). A framework for establishing homologies for defensive circuits in fish, therefore, is useful for this enterprise. Classical neuroscientific approaches to describing behavioral circuits involve the demonstration that a given structure is activated (or inhibited) by specific stimuli or behavioral paradigms, as well as the effects of lesioning (or silencing) and stimulating the structure. Even though there are many technical difficulties in performing functional neuroanatomy in the diminutive zebrafish, novel genetic techniques (Friedrich et al., 2010) have allowed important discoveries regarding the limbic system.

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The increase in the use of zebrafish (Kalueff et al., 2014a) and other species (Hall et al., 2014) as organisms in behavioral models for anxiety- and fear-like behavior also prompted the need to describe the neural structures that are involved in these behaviors (Guo et al., 2012). While the unspoken assumption in the field appears to be one of "multiple realizability" (Bickle, 2010) – including the idea that it is the behavioral function that needs to be similar, and not the brain circuits underlying it, in order to use these assays as models in psychopathology (Wright, 2002) –, at least from an evolutionary point of view it is interesting to assess whether the putative conservation of behavioral responses to aversive stimuli is accompanied by conservation of the neural substratum (Striedter et al., 2014). The present article reviews the circuitry that is involved in detecting, identifying, processing, and responding to threat in fish, with a special focus on zebrafish models.

2. Discovering neural circuits for aversive behavior in fish

An important starting point in determining the aversive behavior network in zebrafish is activation studies. Typically, this is done by examining immediate early gene products, such as the *cfos* gene and its protein, c-Fos. A network comprised of the medial nucleus of the dorsal telencephalon (Dm), ventral nucleus of the ventral telencephalon (Vv), supracommissural nucleus of the ventral telencephalon (Vs), and preoptic region (POA) shows upregulated *cfos* expression after exposure to conspecific alarm substance [CAS] (Faustino et al., 2017). The expression of c-Fos during CAS exposure appears to be specific, as no increases were observed in the lateral nucleus of the dorsal telencephalon (Dl), dorsal nucleus of the ventral telencephalon (Vd), habenula, or thalamus (Ruhl et al., 2017). Dm is also activated after exposure to the light/dark test (Lau et al., 2011; von Trotha et al., 2014), an anxiogenic stimulus for zebrafish (Maximino et al., 2010a). Thus, it appears that a network involving Dm, Vv, Vs, and POA is involved in detecting and/or processing aversive stimuli and/or selecting behavioral strategies in zebrafish. A network for the retrieval of fear memo-

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ries include Dm, Dl, and thalamus, as these structures showed c-Fos expression during presentation of a red light that has been previously associated with CAS (Ruhl et al., 2017).

Other aversive stimuli have also been used to investigate threat detection and processing in zebrafish larvae. A looming stimulus, which simulates predatory attack, activates neurons in the optic tectum (Dunn et al., 2016; Temizer et al., 2015). Tyrosine hydroxylase-positive neurons in the caudal hypothalamus (Hc) respond to aversive stimuli, including pH change, high ammonia, and handling stress (Semenova et al., 2014). Using immunohistochemical detection of phosphorylated extracellular signal-regulated kinase (pERK), Randlett et al. (2015) mapped the activity of nuclei in the larval brain in response to different aversive stimuli (exposure to mustard oil, dish taps, heated water, electric shocks). The authors found that while each stimulus promoted pERK activity in specific regions (due to the diversity of sensory channels tapped by each stimulus), all four activated the locus coeruleus, caudal hindbrain, Hc, POA, and subpallium. It is not clear whether the subpallial regions observed in this experiment overlap with those found in CAS-exposed adults (i.e., Vv and Vs); nonetheless, these results suggest a wider network that is specialized in the detection of aversive stimuli which includes pallial and subpallial components of the amygdala homologues, the neuroendocrine hypothalamus, optic tectum, locus coeruleus, and the brainstem escape network.

The focus of this review will now turn to each of these foci. We will quickly review some of the evidence for proposing these areas as full or partial homologues of specific mammalian nuclei which have been implicated in defensive behavior. Importantly, we will review evidence for the participation of these regions in aversive behavior in fish, and delineate, when possible, the circuitry that is involved in these roles.

3. Pallial and subpallial components of the teleostean amygdala

The fact that the teleostean telencephalon is everted precluded, for a long time, the easy establishment of homologies for telencephalic structures (Nieuwenhuys, 2011). Different eversion

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models lead to different proposed final topologies of pallial structures in adult fish (Braford, 2009; Butler, 2000; Mueller et al., 2011; Nieuwenhuys, 2011; Northcutt, 2008; Wullimann and Mueller, 2004; Yamamoto et al., 2007); as a result, different propositions have been made regarding amygdala homologues in zebrafish. Based on topology and topography, cytoarchitectonics, neurochemistry, expression of developmental regulatory genes, and behavioral data, we will follow our previous proposal that the medial nucleus of the dorsal telencephalon (Dm) is homologous to the “limbic associative” amygdaloid system (lateral and basolateral amygdala of mammals), while the subcommissural and postcommissural nuclei of the ventral telencephalon (Vs and Vp, respectively) are homologous to the “autonomic” amygdaloid system (central extended amygdala [CEXA]) (Maximino et al., 2013a)(Figure 1A).

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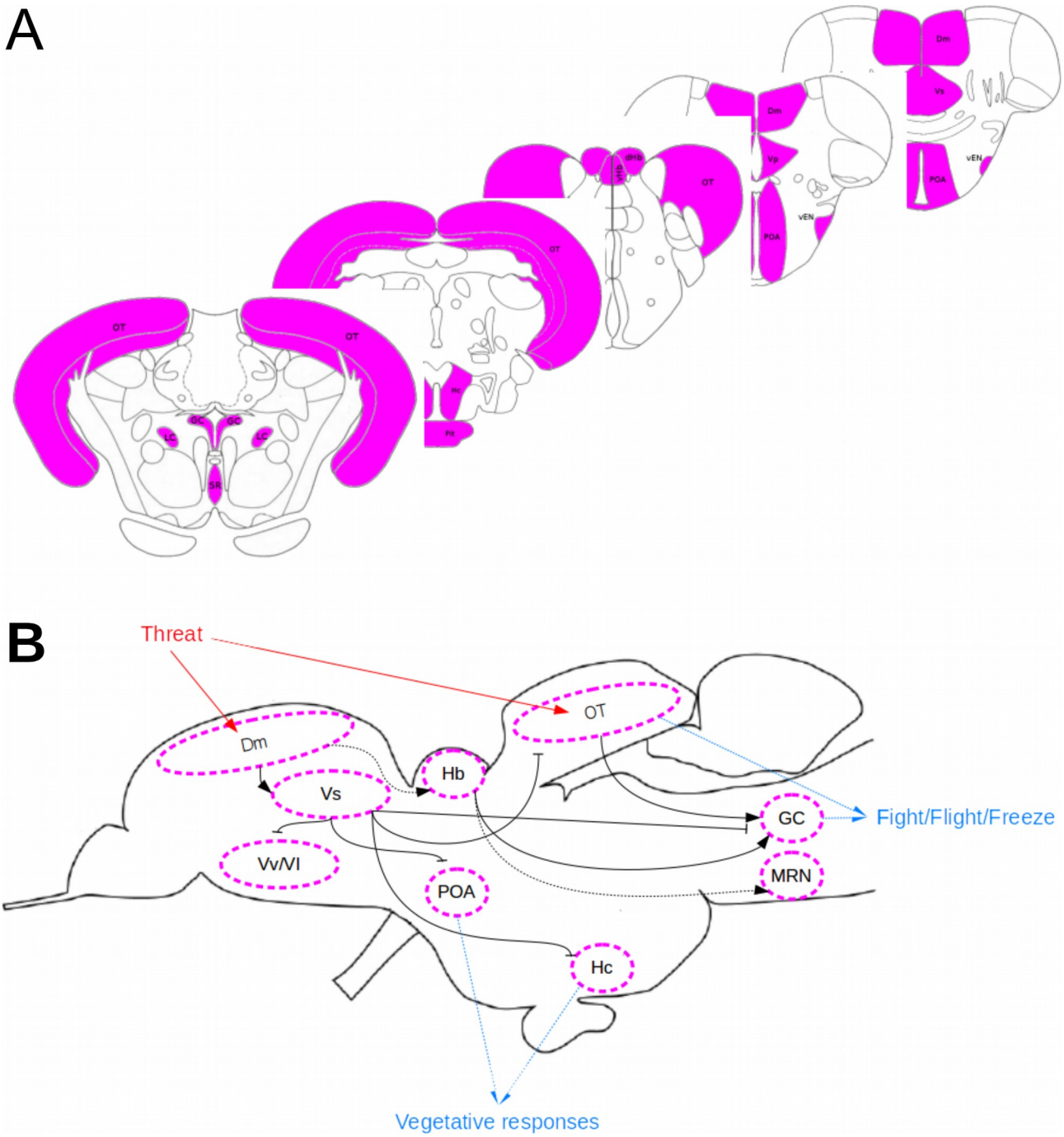

Figure 1: (A) Frontal view of the zebrafish brain circuits for threat detection, identification, and processing, and response selection (in purple), described in Fig. 1B. The right-to-left plans represent the rostrocaudal axis. Schemes adapted from Wulliman et al., 1996. (B) Circuits for threat detection, identification, and processing and response selection in the teleost brain. The dorsomedial telencephalon (Dm) is homologous to the frontotemporal amygdala system, and is the entry for the "forebrain circuit" that terminates in the mesencephalic central gray (GC) and in the hypothalamic circuits for neurovegetative responses (POA, Hc). An indirect projection is also depicted for the ventral habenula (Hb); this projection involves a feedforward glutamatergic projection from the Dm to the entopeduncular nucleus, and from there to the ventral habenula. This can represent part of a habenular circuit in the forebrain circuit which projects indirectly to the raphe (MRN) serotonergic neurons via glutamatergic interneurons in the interpeduncular nucleus and raphe (not shown). The "midbrain circuit" is represented by projections to interneurons in the optic tectum (OT) which mediate fast escape responses via projections to the GC and/or the brainstem escape network (not shown). Threatening stimuli (especially visual stimuli) can bypass the forebrain circuit and directly activate this system, initiating "quick-and-dirty" escape responses.

Abbreviations: Dm: medial nucleus of the dorsal telencephalon; Vs: supracommissural nucleus of the ventral telencephalon; Vv: ventral nucleus of the ventral telencephalon; Vl: lateral nucleus of the ventral telencephalon; Hb: habenula; POA: preoptic area; Hc: caudal hypothalamus; OT: optic tectum; GC: central gray; MRN: median raphe nucleus.

Full black arrows: direct projections; dashed black arrows: indirect projections. Arrows terminating in dimension lines (-┤) represent inhibitory projections. Blue arrows represent outputs, while red arrows represent inputs.

Lesion studies implicated the Dm in associative learning of aversive memories in goldfish. Ablation of the Dm, but not of the Dl, impair the acquisition and maintenance of two-way (active) avoidance acquisition in this species (Portavella et al., 2004b, 2004a, 2002; Portavella and Vargas, 2005). c-Fos studies with zebrafish have also suggested a role for the Dm in aversive conditioning: the association between a red light and CAS produces conditioned responses to the light that are very similar to the innate repertoire of zebrafish (Hall and Suboski, 1995a, 1995b; Ruhl et al., 2017). Interestingly, during the acquisition phase (when the red light and the CAS are presented together), the number of c-Fos-positive cells is higher in the Dm, but not in the lateral nucleus of the dorsal telencephalon (Dl, the hippocampal homologue; Goodson & Kingsbury, 2013) or in the dorsal nucleus of the ventral telencephalon (Vd, the striatum homologue; Ganz et al., 2011). 24 h later, during the retrieval phase (when only the red light is presented), c-Fos-positive cells are increased in the Dm, Dl, and thalamus (Ruhl et al., 2017). In any case, it appears that the Dm is involved in both the acquisition and the maintenance of aversive memories in cyprinids.

The majority of neuro ns in pallial regions are glutamatergic (von Trotha et al., 2014), with some GABAergic interneurons. Lal et al. (2018) produced *Gal4FF* zebrafish lineages driving the expression of the botulinum toxin B light chain (BoTxBLC) gene that showed deficits in active avoidance conditioning. Among these lineages, two showed expression limited to the Dm, and in both the trapped gene was *emx3*. In addition to showing deficits in conditioned aversive behavior, these animals also showed changes in their response to CAS, with decreased freezing but increased erratic swimming. Only 16% of the cells in the Dm express the Gal4FF construct, and the majority are neurons; 94% of these neurons are glutamatergic. These neurons project heavily to the hypothalamus, including anterior tuberal nucleus (ATN), lateral hypothalamic nucleus (LH), and dorsal zone of the periventricular hypothalamus, as well as to telencephalic regions (entopeduncular nucleus, preoptic area, Vd, and Vs)(Figure 1B), suggesting neural networks involved in both conditioned and unconditioned aversive behavior (Lal et al., 2018).

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The role of the Dm in unconditioned behavior has also been assessed. In zebrafish, exposure to the light/dark test increases c-Fos expression in the Dm (Lau et al., 2011; von Trotha et al., 2014). Moreover, CAS exposure also increases *cfos* in the Dm of zebrafish (Faustino et al., 2017), suggesting that ethologically-relevant aversive stimuli recruit these cells even when conditioning is not involved. As already discussed, expression of BoTxBLC in a subset of Dm neurons in zebrafish decreases freezing responses and increases erratic swimming after CAS exposure (Lal et al., 2018). A participation in restraint stress-induced behavioral responses has also been suggested. In Nile tilapia (*Oreochromis niloticus*), restraint stress increases 5-HT metabolism in the Dm (Silva et al., 2014). Likewise, acute stress (lowering water levels) increases *htr1aa* and *htr1ab* expression in the Dm of rainbow trout (Vindas et al., 2017); interestingly, 5-HIAA levels were increased in the Dm only in those animals which were classified as "proactive" in relation to a behavioral response to hypoxia (Vindas et al., 2017), suggesting that 5-HT acts in this nucleus to mount active responses to remove stressors and restore homeostasis. Moreover, destroying 5-HT fibers in the telencephalon with the serotonergic toxin 5-7-DHT decreases the ratio of correct avoidance responses in an active avoidance paradigm in zebrafish (Amo et al., 2014). Thus, feedback mechanisms from neuromodulators appear to also be important in these responses (Table 1). Consistent with the hypothesis of a role of the Dm in active responses to aversive stimuli, inhibition of the Dm in *Leporinus macrocephalus* by injecting midazolam, an anxiolytic and sedative benzodiazepine (Dundee et al. 1985), abolished stress-induced analgesia (Wolkers et al., 2015).

Table 1 – Neuromodulatory (monoaminergic) innervation of nuclei in the aversive brain system of teleosts. Given that monoaminergic regions such as the raphe are important targets of forebrain and midbrain circuits, it is likely that modulating the activity of these neuromodulators represent ways to "fine-tune" defensive responses, including by feedback. The number of '+' symbols represent the relative amount of innervation in these regions.

Abbreviations: 5-HT: serotonin; DA: Dopamine; NE: noradrenaline; Dm: medial nucleus of the dorsal telencephalon; Vs: supracommissural nucleus of the ventral telencephalon; Vv: ventral nucleus of the ventral telencephalon; Vl: lat-

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eral nucleus of the ventral telencephalon; vEN: ventral entopeduncular nucleus; Hb: habenula; POA: preoptic area; Hc: caudal hypothalamus; OT: optic tectum; GC: central gray.

	5-HT innervation	DA innervation	NE innervation
Dm	+	0	
Vs	+	+	++
Vv	+	++	++
Vl	+	+	++
vEN	++	0	?
Hb	++	0	0
POA	+++	++	+
Hc	+++	+++	+
OT	++	++	+
GC	+++	?	?

In rodents, different components of the limbic associative amygdalar system mediate responses innate stimuli (auditory and visual components of predator cues) vs. conditioned stimuli (i.e., stimuli which were previously paired with noxious stimuli)(Gross and Canteras, 2012). In mammals, olfactory components of predator cues bypass the cortical components of the amygdala, instead projecting directly to subcortical components (posteroventral part of the medial amygdala; Gross and Canteras, 2012). These different components process distinct afferent inputs and channel them to parallel downstream efferent pathways, responsible for output circuits for fear of predators and fear of pain (Gross and Canteras, 2012). In zebrafish, sensory input to the posterior telencephalon (including Dm) suggest massive olfactory information (e.g., Miyasaka et al., 2014), while visual and auditory stimuli are processed initially in the optic tectum (Northcutt, 1983; Meek, 1990); however, the fact that an olfactory stimulus can be associated with a visual stimulus in the Dm (Ruhl et al., 2017; Lal et al., 2018) suggests the participation of a projection from the optic tectum, possibly via the lateral preglomerular nucleus (Carr, 2015). Thus, in zebrafish the Dm also appears to be important for learned fear, but the participation in unconditioned behavior suggests that either subpopulations of the Dm process different stimuli, or that the evolutionary shift towards vis-

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ual and auditory stimuli in mammals displaced the massive olfactory projections that was found in fish.

If there is considerable evidence for the homology between Dm and the limbic associative amygdala of mammals, this is not the case for the homology between Vs/Vp and the autonomic amygdala; in fact, it is more probable that these regions are only *partially* homologous to the CEXA (Goodson and Kingsbury, 2013; Maximino et al., 2013a). In mammals, the CEXA has been proposed to mediate the selection of vegetative and behavioral responses to threatening stimuli (Gozzi et al., 2010; LeDoux and Pine, 2016), as well as conditioned fear (Ciocchi et al., 2010; Haubensak et al., 2010; LeDoux, 1998). The circuit acts by tonically inhibiting downstream responses mediated by the periaqueductal gray area (PAG) and hypothalamus (freezing, flight), or by the basal forebrain (risk assessment); inhibition of these circuits (by, e.g., signals from the "limbic associative" amygdala) release behavior in one of these two streams, producing appropriate active or passive responses to the threatening stimulus (Maximino, 2012). In zebrafish, these neurons are also GABAergic (Mueller and Guo, 2009), suggesting a similar circuit.

Almost no behavioral evidence exists for the role of Vs/Vp in defensive behavior. One of the possible reasons is that the Vs and Vp are continuous with the Vd in a rostrocaudal axis, leading authors to report effects (or lack thereof) of interventions in wrongly identified structures. One important exception is the observation that CAS increases *cfos* expression in the Vs (Faustino et al., 2017). Interestingly, as reported above, this work also found increased *cfos* in the Dm, Vv, and POA. Importantly, control animals showed functional connectivity between these regions, while exposure to CAS decreased these correlations; in fact, animals exposed to CAS showed only co-activation of Dm-Vs and Vv-Vs (Faustino et al., 2017). The precise mechanism for this decreased coherence is yet to be described; however, considering the equivalent mammalian circuit, it is possible that activation of the Dm activates inhibitory neurons from the Vs, which could be responsible for

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196 decreased connectivity with Vv and POA, while at the same time disinhibiting downstream (hy-
197 pothalamic and mesencephalic) mechanisms for response emission (Figure 1B).

198 In summary, the Dm appears to be important for both conditioned and unconditioned aver-
199 sive responses, as well as to mounting appropriate (active vs. passive) responses. The Vs also ap-
200 pears to participate in processing unconditioned threat, but its role in response selection and learn-
201 ing is still unknown.

202

203 **3. Does the habenula participate in defense?**

204 The habenulae are paired structures located in the roof of the rostral diencephalon of fishes,
205 divided classically into a dorsal, asymmetric portion (dHb) and a ventral, symmetric portion (vHb).
206 The dHb can be further subvidided into medial and lateral subnuclei (Aizawa et al., 2011; Okamoto
207 et al., 2011, 2008), although eleven subdivisions (Hb01 - Hb10) are suggested by single-cell RNA-
208 Seq registered to anatomy (Pandey et al., 2018). Four clusters were identified in the zebrafish vHb
209 (Hb11, Hb12, Hb13, and Hb15), based on the expression of genetic markers and signaling
210 molecules (Pandey et al., 2018). The vHb receives (putatively glutamatergic) projections from the
211 ventral portion of the entopeduncular nucleus (vEN) (Okamoto et al., 2011; Amo et al., 2014),
212 which by its turn receive excitatory projections from the Dm (Lal et al., 2018); as a result, a
213 excitatory feedforward circuit exists from Dm to vEN to vHb.

214 The difference in size between left and right habenulae is due mainly to the enlargement of
215 the lateral subnucleus (dHbL) in the left habenula in relation to the right, which shows an enlarged
216 medial subnucleus (dHbM) (Amo et al., 2010; Okamoto et al., 2011). This difference correlates with
217 parapineal asymmetry (Gamse et al., 2003) and is concordant with the lateralization of viscera
218 (Barth et al., 2005; Domenichini et al., 2011) and subsets of behavioral functions (Barth et al., 2005;
219 Dadda et al., 2010; Facchin et al., 2009) – including “binary” opposing strategies such as escape
220 and freezing –, whose processing seems to be guaranteed by the asymmetrical structure DHb

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thought to have evolved under the natural selection pressure resulting from functional incompatibility between these behaviors (Ichijo et al. 2017). In the left dHb, high levels of the potassium channel tetramerization domain-containing protein 12.1 (*kctd12.1/leftover/lov*) are expressed, while low levels are observed in the right dHb. Conversely, two other members of the KTCD family, *kctd12.2* (*right on/ron*) and *kctd8* (*dexter/dex*), are expressed exclusively in the right dHb (Beretta et al., 2012; Gamse et al., 2005; Y. Kuan et al., 2007; Roussigné et al., 2011). High levels of *murcb* and the adrenoceptor beta 2 *adrb2a* are found in clusters enriched in the right dHb, while clusters enriched in the left dHb show high levels of the protochaderin *pcdh7b*, the Wnt family member *wnt7aa*, adenylate cyclase-activating polypeptide *adcyap1a*, and the protein phosphatase regulatory inhibitor *ppp1r1c* (Pandey et al., 2018). These genes have been used as markers for the neuroanatomical divisions of the dHb. However, caution should be exercised in transposing these neuroanatomical subdivisions to functional ones: DeCarvalho et al. (2014) showed that cholinergic neurons in the left dHb are a distinct population from cells expressing *kctd12.1*, and part of the developmental expansion of the lateral dHb from larvae to adults is accompanied by the invasion of the left dHb with cholinergic neurons (in larvae, most neurons in the left dHb are glutamatergic only) and an expansion of substance P and somatostatin-expressing neurons.

While the dHb is asymmetric, the ventral habenula (vHb) is symmetric (Amo et al., 2010; Okamoto et al., 2011). The vHb is characterized by the expression of *diamine oxidase* (*dao*), *kiss1* and *protocadherin 10a* (*pcdh10a*) mRNA (Amo et al., 2010; Beretta et al., 2013). Cluster Hb11 is marked by high expression of the transcription factor *sox1a*, cluster Hb12 by high expression of the 5-HT_{1A} receptor *htr1aa*, cluster Hb13 by high expression of *beta-tubulin 5* (*tubb5*), and cluster Hb15 by the high expression of the kisspeptin gene *kiss1* (Pandey et al., 2018). Again, these genes have been used as markers for the neuroanatomical subdivision of the vHb.

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The neuroanatomical asymmetry of the dHb has produced an interesting literature on its behavioral correlates, especially in zebrafish. The neuroanatomical asymmetry correlates with behavioral asymmetries in different assays. Animals with left parapineal position (L-PPO) tend to use their right eye when viewing a mirror and swim preferentially in a clockwise direction, while animals with right parapineal (R-PPO) do not show this preference; conversely, R-PPO zebrafish use the right eye to inspect a live predator, while L-PPO animals show no preference (Taylor et al., 2011). Importantly, while these animals show preference for eye use for a stimulus which elicits aggression (mirror image) or defensive behavior (predator), no eye preference whatsoever is observed towards “neutral” stimuli (Y.-S. Kuan et al., 2007) In addition to the lateralization of behavior, some important differences are observed in adult behavior in other domains. In the first minutes of an open tank test, R-PPO fish show less thigmotaxis than L-PPO fish; likewise, R-PPO fish spend more time near a predator towards the end of the task (Aizawa et al., 2011; Beretta et al., 2012; Concha et al., 2009; Okamoto et al., 2011). In the *frequent-situs-inversus* lineage, larvae with a left-lateralized habenula (LH) begin to view their mirror image with the right eye, but then change to the left eye by the end of a five-minute period; in larvae with a right-lateralized habenula (RH), this pattern is reversed (Dadda et al., 2010; Domenichini et al., 2011). When adults are confronted with a two-choice bead test, *frequent-situs-inversus* lineage fish with a left-lateralized habenula fish (LH *fsi*) bite the right target, while RH *fsi* fish bite the left target. In an emergence test, when LH *fsi* fish are confronted with a black stripe they show progressively increasing latencies to change compartment; while this effect is observed in RH *fsi* fish, it is much smaller (Barth et al., 2005). These results seem to suggest that a right-lateralized habenula decreases responsiveness to novel and threatening stimuli.

Contrary to this hypothesis, however, zebrafish expressing the tetanus toxin light chain in the dHbL [Tg(*narp:GAL4*^{VP16}; UAS:TeTxLC) lineage] show more bottom-dwelling in a novel tank, as well as increased freezing responses to an alarm substance combined with the presentation of a

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moving shadow above the tank (Mathuru and Jesuthasan, 2013); nonetheless, *c-fos* or *egr1* expression is not significantly changed in the dHb after exposure to an alarm substance in zebrafish (DeCarvalho et al., 2013). On the other hand, in larvae, expression of botullin toxin light chain [Tg(*gng8: Gal4*; UAS:BoTxBLC-GFP)] in the dHbL (but not in the dHbR) impairs light preference (Zhang et al., 2017), consistent with decreased anxiety (Steenbergen et al., 2011). Adult animals with silenced dHbL show increased dark preference (Zhang et al., 2017), consistent with *increased* anxiety (Maximino et al., 2010a). Cheng et al. (2017) demonstrated in larval zebrafish habenular responses to light "on" and "off" responses are mediated by the nucleus in the anterior thalamus. This nucleus innervates the dorsal left neuropil of the habenula and converges information about environmental illumination because it receives afferents from the retina and pineal. This thalamo-habenula projection seems to be involved in the ability of blue light to mask circadian variations in behavior (Lin and Jesuthasan, 2017). The role of this thalamo-habenular pathway on light/dark preference, however, is still unknown. Moreover, exposure to the light/dark assay, a model of anxiety-like behavior, increased *cfos* mRNA expression in the dHb of zebrafish, but only if they are handled before the experiment (Lau et al., 2011). A subset of neurons in the dHbL, expressing the myosin phosphatase Rho-interacting protein *mprip*, shows increased *cfos* mRNA expression after an electric shock (Pandey et al., 2018). These results suggest that the zebrafish dHbL is an important center in defensive behavior, being necessary to mount an adaptive response to innate aversive stimuli; in adults, dHbL appears to inhibit anxiety- and fear-like behavior, while in larvae it appears to increase it.

Other important pharmacogenetic work attempted to establish the role of the habenula in stimulus appraisal and behavioral control in zebrafish. In the first work, Agetsuma and colleagues (2010) expressed the tetanus toxin light chain [Tg(*narp:GAL4*^{VP16}*; UAS:TeTxLC*)] or a nitroreductase-mCherry fusion protein in dHbL neurons, and were thus able to block synaptic transmission in that area. When they reached maturity, animals were trained in a cued fear

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conditioning task; while controls showed increased flight behaviors to the cue after conditioning, dHbL-silenced fish instead showed persistent freezing to the cue. In another experiment, Lee et al. (2010) expressed the photo-sensitizer KillerRed in a ventral telencephalon-habenula projection, and photobleaching on the left habenula led to deficits in the acquisition of two-way avoidance and hyperarousal after light onset before conditioning. They also expressed TeTxLC in the dHbL, obtaining the same result regarding avoidance conditioning deficit.

Interestingly, the dHbL has also been implicated in social conflict resolution. Chou et al. (2016) showed that, after the establishment of social dominance, loser (socially submissive) fish show intense activity in the ventral IPN and median raphe and a very reduced responsiveness of the dorsal IPN after acute electrical stimulation of the Hb. These results suggested a participation of different dHb-IPN pathways in the behavioral plasticity that is associated with the losing experience. Indeed, zebrafish expressing the tetanus neurotoxin in the dHbM [Tg*(gpr151:GAL4VP16; brn3a-hsp70:GFP-Cre; UAS:loxP-DsRed-loxP-GFP-TeNT)*] show a consistent trend to win fights, while dHbL-silenced [Tg*(narp:GALVP16; UAS:TeNT)*] fish showed a consistent trend to lose fights (Chou et al., 2016). The authors proposed that the reduction in the dHbL-iIPN-GC pathway could switch behavior from offensive behavior to defensive behavior (i.e., from attacking to fleeing), resulting in losing the fight. Conversely, reduction in the dHbM-vIPN-MRN pathway results in winning the fight by disinhibiting the MRN, which would tend to increase resilience to aversive stimuli.

The role of the vHb has been less extensively studied. An interesting exception is the work of Amo et al. (2014), which demonstrated two types of responses in vHb neurons after fear conditioning. One type responds phasically to an unconditioned stimulus (US) before conditioning, a response which is gradually substituted by sustained firing for all duration of the presentation of the conditioned stimulus (CS). A second type responds phasically to the US and gradually substitute this response to phasic firing to the CS. Amo et al. (2014) suggested that these responses code for

aversive expectation value and prediction error, respectively. Multi-unit activity of the vHb increases in the early stages of active avoidance conditioning and later returns to normal, suggesting that the vHb encodes the negative reward expectation value in active avoidance learning (Amo et al., 2014). Expression of TeTxLC in the vHb abolishes active avoidance learning without alterations in basal anxiety-like behavior or fear conditioning, strengthening the hypothesis of a specific role in active avoidance conditioning; finally, pairing the optogenetic activation of the vHb with a specific tank compartment elicits avoidance of that compartment only when the stimulation is tonic, but not when it is phasic. Overall, these results suggest that tonic responses in the zebrafish vHb represent an aversive expectation value, participating in a larger vEP-vHb-MRN circuit (Amo et al., 2014).

Another important evidence for the role of the vHb comes from work with kisspeptin, a system of peptides associated with reproductive behavior. In zebrafish, the neuropeptide Kiss1 is expressed only in the vHb, while Kiss2 is expressed in the hypothalamus (Servili et al., 2011). These Kiss1-positive co-express glutamate, and project to glutamatergic interneurons in the IPN and MRN (Nathan et al., 2015a). Interestingly, kissr1 receptors are not found in the raphe, but can be found in the vHb and the IPN (Ogawa et al., 2012). Intracranial administration of Kiss1 increases c-Fos expression in the vHb and MRN, and increases the expression of serotonergic system-related genes (*pet1* and *slc6a4a*) (Ogawa et al., 2014, 2012). It is not known whether CAS induces c-Fos expression in the vHb, since DeCarvalho et al. (2013) only reported results from the dHb. Kiss1 also induces a dose-dependent effect on vHb neurons, with low concentrations leading to depolarization and high concentrations leading to hyperpolarization (Lupton et al, 2017). Importantly, Kiss1 blocks responses to CAS (Ogawa et al., 2014), an effect which is mediated by 5-HT_{1A} and 5-HT_2 receptors (Nathan et al., 2015b). Paradoxically, Kiss1 mutant zebrafish with a stop codon upstream of the active peptide show impaired performance in an active avoidance task (Lupton et al., 2017). Interestingly, Kiss1 mutants also show blunted calcium responses in the superior raphe after an electric shock (Lupton et al., 2017). These results underline a mechanism by which the vHb-MRN cir-

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cuit works: aversive stimuli activate Kiss1/glutamatergic neurons in the vHb, which project to excitatory interneurons in the IPN and raphe. This feed-forward mechanism induces the activation of the raphe, encoding expectations of dangerous outcomes. These expectations can be compared to real outcomes by the activation of 5-HT_{1A} and 5-HT_2 receptors, which have been shown to be important in controlling aversive behavior in zebrafish (Maximino et al., 2014, 2013b; Nowicki et al., 2014). Lupton et al. (2017) suggested that this excitatory stage, associated with increased aversive expectation, favors CS-US association, while the next stage in avoidance learning is mediated by kisspeptin-evoked inhibition of the habenula and consequent reduced aversive expectations once the strategy to avoid the US has been learned.

There is still little work on the interactions between the dorsal and ventral portions of the habenula in aversive reactions. The different functional roles of these circuits, however, suggest some complementarity. While the ventral aspects appear to be involved in coding aversive value – especially through the vHb-raphe pathway –, the dorsal pathway appears to be involved in shifting behavioral strategies from active to passive coping. It is highly likely that this “switch” function depends on information on aversive value and expectations; whether this is provided directly by vHb-dHb connections, or indirectly, by a feedback loop organized by the raphe, is so far unknown. Moreover, this is likely an oversimplification of the role of the habenula in a larger circuit that includes the telencephalon and other portions of the aversive brain system.

4. Hypothalamic circuits for defense

The hypothalamus of teleosts fish presents pair of ventrolaterally extending hypothalamic lobes. These lobes ranges from moderately elevated lobes up to hemisphere-like corpora reaching almost the size of the optic tectum (Senn, 1981). The use of diverse model systems to study hypothalamus development has provided evidence that the molecular pathways regulating hypothalamic induction and patterning are generally conserved from fish to mammals. In addition, the basic hy-

pothalamic cell types and the codes of gene expression that specify them are also highly homologous throughout vertebrate species (Xie and Dorsky, 2017). The fish hypothalamus contains equivalents to most if not all of the mammalian hypothalamic cell types. The hypothalamic neurons are all located in stereotypical clusters within the ventral diencephalon hypothalamic and neuronal populations that control the pituitary in fish have been conclusively shown to be functionally analogous to their mammalian counterparts (Machluf et al., 2011).

In teleost fish, partial homologies for hypothalamic nuclei that are relevant for defensive behavior have been proposed: the preoptic area (POA), for example, is composed of cells of different sizes that form clusters considered homologous to mammalian hypothalamic nuclei (Goodson and Kingsbury, 2013). Groups of magnocellular and gigantocellular cells are considered homologous to the supraoptic nucleus, while the cluster of parvocellular cells is supposed to be homologous to the paraventricular nucleus (Moore and Lowry, 1998). Between the POA and ventral hypothalamic region there is a transition zone called ventral tuberal region (VTN) that is thought as homologous to the mammalian anterior hypothalamus (AH), and in the ventrocaudal part of the hypothalamus is located the ATN, a putative homolog of the mammalian VMH (Forlano et al., 2005; Forlano and Bass, 2011; Goodson, 2005; O'Connell and Hofmann, 2011). The AH is a source of vasopressin in the mammalian brain, and activation of these vasopressinergic neurons increases aggressive behavior in rodents (Gobrogge et al., 2007).

Hodology also supports the homology of these regions. Both the ATN Several hypothalamic regions of teleosts, including ATN and VTN, are connected to the Vs (Folgueira et al., 2004), which has been proposed as homologous to the medial amygdala (Biechl et al., 2017) or to the extended central amygdala (Maximino et al., 2013a) – regions which, as discussed above, participate in the processing of aversive stimuli and defensive responses of both mammals and teleosts. Thus, it is possible that these connections of hypothalamic regions to Vs are related to regulation of defensive behavior in fish.

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Unfortunately, no functional studies have been made assessing the role of the ATN and VTN in behavioral responses. In teleosts, arginine vasotocin (AVT, the homologue to vasopressin) is also expressed in the VTN (Rodriguez-Santiago et al., 2017), and manipulating AVT levels decreases antipredator behavior in zebrafish (Braida et al., 2012). However, AVT is also expressed in other brain regions, including the POA and pallial and subpallial amygdala (Rodriguez-Santiago et al., 2017), precluding any speculation as to the role of the VTN. The ATN of teleosts also expresses sex steroid hormone receptors, as is the case of the VTN (O'Connell and Hofmann, 2012). Stimulation of the ATN elicits reproduction-related vocalizations in male midshipman fish *Porichthys notatus* (Goodson and Bass, 2000), suggesting a role in reproductive behavior; however, a role in defensive behavior has not yet been determined.

Indirect evidence for a participation of the hypothalamus in defensive behavior is stronger in relation to neuroendocrine endpoints, especially cortisol responses, given that these responses are under descending hypothalamic control. Aversive stimuli which have been shown to elicit cortisol responses in zebrafish include acute chasing stress (de Abreu et al., 2016; Idalencio et al., 2017; Tran et al., 2014), acute restraint stress (Abreu et al., 2017a; Ghisleni et al., 2012), unpredictable chronic stress (Piato et al., 2011), exposure to the novel tank or the light/dark test (Kysil et al., 2017), and subordinate-dominant interactions (Pavlidis et al., 2011). CAS also elicits increases in cortisol responses in Nile tilapia (Silva et al., 2015) and zebrafish (Abreu et al., 2017a; Schirmer et al., 2013); interestingly, cortisol responses are also observed after visual contact with a predator in zebrafish (Barcellos et al., 2010, 2007), and *D. rerio* also appears to be able to communicate predation risk to conspecifics, since cortisol responses are observed after seeing a shoalmate displaying antipredator behavior (Oliveira et al., 2017, 2013).

Presently little is known about either a causal relationship between cortisol and behavioral responses to aversive stimuli in fish, or about which hypothalamic regions are involved in these responses. A clue comes from Ziv et al. (2013) and Griffiths et al. (2012), which used a mutant

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zebrafish lineage (*gr*^{s357}) with non-functional glucocorticoid receptors. Larvae from this line show elevated whole-body cortisol levels, increased expression of pro-opiomelanocortin, and no suppression of stress markers after dexamethasone treatment (Griffiths et al., 2012). These larvae also show more auditory-evoked startle responses when compared to wild-type controls, an phenotype that is rescued by treatment with fluoxetine (Griffiths et al., 2012). Adult *gr*^{s357} animals show abnormal freezing behavior when introduced to a novel tank, reduced exploratory behavior, and lack of habituation to environmental novelty (Ziv et al., 2013); chronic treatment with fluoxetine rescue this phenotype and suppresses the stress-induced upregulation of the mineralocorticoid receptor and the serotonin transporter *scl6a4a* (Ziv et al., 2013). These results suggest a developmental role for the glucocorticoid receptor on shaping the serotonergic system and, as a consequence, increasing anxiety-like behavior in both larvae and adults. The acute effect of cortisol is unknown in this species; in goldfish, cortisol rapidly increases Mauthner cell excitability (Bronson and Preuss, 2017), suggesting an acute, non-genomic mechanism to facilitate the brainstem escape network (see below). Finally, in the crucian carp *Carassius carassius*, the CRF_1 receptor antagonist antalarmin suppresses CAS-elicited responses (Lastein et al., 2008), suggesting a role for CRF in these responses; however, it is not known whether this response is mediated by the hypothalamus or by extra-hypothalamic sites, such as the Dm.

5. Tectal circuits for detection of visual threatening stimuli

The most prominent structures within the fish tectum are the optic tectum (TO) and torus semicircularis (TS); they are homologous to the mammalian superior and inferior colliculi, respectively (Nieuwenhuys et al., 1998). Zebrafish present at least six easily identifiable tectal layers (from superficial to deeper: marginal [MS], optic [SO], superficial fibrous and gray [SFGS], central gray [SGC], central white [SAC], and periventricular strata [SPV]). It has been observed that, in all vertebrates, the upper layers of the tectum are retinorecipient, while the deeper layers house the pro-

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jection neurons (Butler and Hodos, 2005). In rodents, information from the upper visual field is represented in the medial optic tectum, while information from the lower visual field is represented in the lateral portion; likewise, stimulation of the lateral portion leads to approach-like and appetitive movements, while stimulation of the medial portion leads to defensive-like behavior (Brandão et al., 2003, 1999). In rodents, these medial regions receive exclusive projections from multimodal and association sensory cortices, visual thalami, hypothalamic nuclei associated with defensive behavior, and a few pretectal nuclei (Comoli et al., 2012). In goldfish, the medial tectal zone seems to be related to orienting responses, the anteromedial zone to goal-directed saccades, the extreme anteromedial zone to eye convergence, and the posterior zone to escape responses (Herrero et al., 1998; Salas et al., 1997).

Many different interneurons have been described in the OT (Figure 2A). Superficial interneurons (SINs) in the superficial layers (SO and SFGS) are GABAergic (Scott and Baier, 2009). About 75% of the cells from the periventricular stratum (SPV) are GABAergic, while 10% are glutamatergic (Nevin et al., 2010; Robles et al., 2011). Glutamatergic cells from the tectum are of the bistratified periventricular (bsPVIN) interneuron type, with somata which locate in the deeper and intermediate regions of the SPV, a single apical process that spans the SGC and SFGS, and glutamatergic axons which terminate in the layer between both of these strata (Nevin et al., 2010; Robles et al., 2011). Another group of cells, the non-stratified periventricular (nsPVIN) type, is a small population of GABAergic interneurons located deep in the SPV, arbor in the deeper regions of the SGC and SFGS; they lack the stratification and laminar specificity of bsPVIN cells, with their axons terminating mostly in the SGC and in between this stratum and the overlying SFGS and the underlying SAC (Nevin et al., 2010; Robles et al., 2011; Scott and Baier, 2009). Finally, periventricular projection neurons (PVPNs) are GABAergic cells with a dendritic arbor spanning the regions between SFGS and SGC, the SGC itself, and the region between SGC and SAC, and an axon that forms a sparse arbor of collaterals in the vicinity of the lateral longitudinal fascicle and the hind-

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brain escape network (Nevin et al., 2010; Robles et al., 2011; Scott and Baier, 2009). Some periventricular projection cells course medially and terminate in the superior raphe (Nevin et al., 2010), but it is not known whether these cells are GABAergic or not; however, lipophilic dye injection into the tectal neuropil marks a much larger population of axons than the GABAergic neurons, suggesting that this is a small and very specialized projection (Nevin et al., 2010; Scott and Baier, 2009).

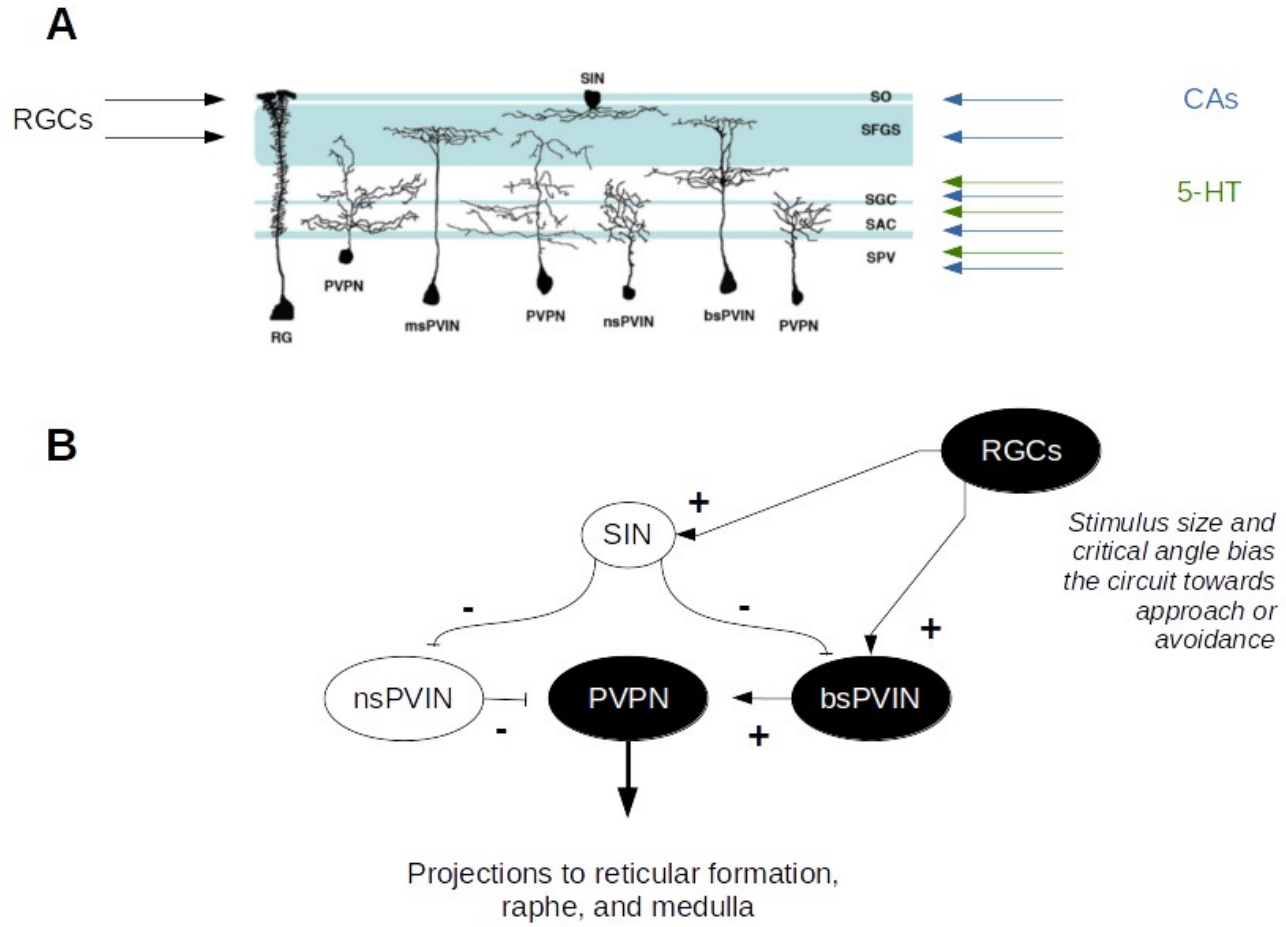

Figure 2: (A) Depiction of the tectal interneurons that participate in the escape circuit. The upper layers of the tectum (stratum opticum and stratum fibrosum et griseum superficiale) are retinorecipient, and also receive catecholaminergic (CAs) projections. Interneurons in the deeper layers (stratum griseum centrale, stratum album centrale, stratum periventriculare) participate in decision-making, and receive serotonergic (5-HT) and catecholaminergic projections. Identified interneuron types include the superficial inhibitory interneuron (SIN), as well as a variety of periventricular interneurons (non-stratified [nsPVIN), bistratified [bsPVIN], and mono-stratified [msPVIN] interneurons). Morphologically distinct periventricular projection neurons (PVPNs) have also been identified. (B) Stimulus properties, including size, are coded by retinal ganglion cells (RGCs) bias the circuit towards approach (smaller size) or avoidance (large size). (B) These stimuli activate inhibitory SINs and excitatory bsPVINs. SINs also fine-tune responses of bsPVINs, and disinhibit PVPNs by inhibiting nsPVINs. These projection neurons, in its turn, modulate the activity of the reticular formation, raphe, and medulla.

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bsPVINs, nsPVINs and PVPNs have been thought of as representing different parts of a circuit for detecting visual threats and selecting appropriate responses (Fig. 2B). The distribution of the neuropil, as well as the glutamatergic nature of the bsPVIN neuron type suggest that these cells perform superficial to deep information transfer (Nevin et al., 2010; Robles et al., 2011). In contrast, the inhibitory nsPVIN is a GABAergic interneuron, producing feed-forward inhibition to filter the visual information transmitted onto projection neurons (Robles et al., 2011). Moreover, the location of their dendrites primarily in the SGC layer, which receives non-visual afferents from the telencephalon and thalamus, suggest that they might integrate inputs from visual and non-visual areas (Nevin et al., 2010). Since projection cells from the tectum have dendrites in the deeper and intermediate layers of neuropil (but not the superficial layers), this suggests that they are not directly retinorecipient (Butler and Hodos, 2005), and therefore must receive information from bsPVIN and nsPVIN cells.

In zebrafish, the role of these circuits in escape responses has been described in a series of elegant experiments in larvae. Zebrafish larvae respond to a moving dot stimulus in a size-dependent way: if the stimulus is relatively small (e.g. potential prey), the animal approaches it, while larger stimuli (e.g., potential predators) are avoided (Barker and Baier, 2015). Silencing tectal neurons completely abolishes both approach and avoidance responses, irrespective of stimulus size. When SINs are ablated, however, large object avoidance is impaired (Barker and Baier, 2015). Interestingly, ablating tectal cells marked in the *Gal4mpn354* line – most of which are glutamatergic nsPVINs – shift behavior from approach to avoidance, with ablated larvae avoiding small stimuli; optogenetic stimulation of these neurons produce the opposite effect (Barker and Baier, 2015). Noteworthy, larvae acutely treated with fluoxetine, therefore increasing serotonergic tone, decrease the probability of avoiding small and medium stimuli, but do not affect responses to larger stimuli (Filosa et al., 2016), suggesting that this neurotransmitter does not participate in the control of escape responses by the OT, but instead participates in shifting from fleeing to foraging.

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Using a different stimulus – a "looming" dot that increases in size, simulating a predator strike – it was demonstrated that zebrafish larvae attempt to escape this stimulus (Dunn et al., 2016; Temizer et al., 2015), and that this stimulus activates three specific targets of retinal ganglion cells: arborization fields AF6 and AF8, and the OT (Dunn et al., 2016; Temizer et al., 2015). In the OT, responses were observed in the SFGS and SGS (Temizer et al., 2015); a specific response is observed in SINs (Dunn et al., 2016). It appears that these SINs modulate the inputs from retinal ganglion cells to periventicular projection neurons, "fine-tuning" the motor command produced by these later cells to critical angle (Dunn et al., 2016). The higher density of looming-selective responses in the OT in relation to extra-tectal retinorecipient areas suggest that the OT is involved in processing looming stimuli, while AF6 and AF8 process other visual cues such as whole-field motion and luminance changes. Indeed, ablation of retinotectal projections – leaving intact projections to other AFs – impaired the ability of larvae to escape the looming stimulus (Temizer et al., 2015). Ablation of the brainstem escape network – one important motor output from OT – resulted in a specific bend deficit in response to looming stimuli, suggesting a participation of these neurons in the escape response elicited by looming stimuli (Dunn et al., 2016).

6. The brainstem escape network

The concept of a "brainstem escape network" was introduced to describe gigantocellular neurons in the brainstem of fish (the Mauthner [M-]cells, its two segmental homologs MiD2cm and MiD3cm, and other identified neurons in the reticulospinal segments adjacent to the Mauthner cells) that activate fast-start responses that are used by fish to escape predatory attacks (Eaton et al., 2001). These reticulospinal system receives massive primary acoustic input as well as sensory inputs from the optic tectum, and synapses on motoneurons that innervate trunk muscle on the contralateral side (Kinkhabwala et al., 2010). The activation of M-cells produces a robust turn of about 45º, leading to the initiation of a very fast response called C-start (Furukawa and Furshpan, 1963;

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Eaton et al., 1977) that is fine-tuned to the angle of stimulation; the participation of the other components of the brainstem escape network code other kinematic features that result in propelling the fish away from the stimulus (Eaton et al., 2001). These responses are very fast; in zebrafish, the latency for a C-start after acoustic stimulation was recorded as about 5 ms (Eaton et al., 1977), and the latency for looming visual stimuli varied from 10-20 ms (Temizer et al., 2015) to a few hundreds of milliseconds (Dunn et al., 2016).

The brainstem escape network receives inputs from many different regions of the teleostean brain. As described, escape responses to visual stimuli are mediated by the optic tectum (Dunn et al., 2016; Temizer et al., 2015), which projects to this system. While telencephalic projections have not yet been described, telencephalic ablation decreases startle probability in goldfish (Collins and Waldeck, 2006), suggesting a facilitatory role; whether this is due to ablation of pallial or subpallial amygdalar components is unkown.

Monoaminergic inputs are also important in the modulation of C-starts. In zebrafish larvae, tyrosine hydroxylase and 5-HT immunoreactivity was observed closely apposed to the ventral dendrites of the M-cell, MiD2cm, and MiD3cm, and tyrosine hydroxylase immunoreactivty was observed near the lateral dendrite (McLean and Fetcho, 2004). 5-HT increases inhibitory currents produced by activation of presynaptic pathways, while dopamine increases the amplitudes of electrical and glutamatergic components of auditorily evoked responses (Korn and Faber, 2005).

While this apparently simple reflex has been considered to be a “fixed action pattern”, with little modulation by upstream structures and therefore little relevance for fear- and anxiety-like states, there is interesting evidence for considerable plasticity of this system (Medan and Preuss, 2014). Larval zebrafish present prepulse inhibition (PPI), in which the probability of a C-start is reduced when it is preceded by a non-startling sound (Burgess and Granato, 2007). This prepulse sound attenuates the synaptic response of M-cells to a subsequent auditory stimulus (Curtin et al., 2013; Medan and Preuss, 2014, 2011). In zebrafish, the PPI is disrupted by apomorphine, a

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dopaminergic agonist (Burgess and Granato, 2007); in goldfish, apomorphine blocks the prepulse sound-evoked reduction in M-cell membrane resistance (Medan and Preuss, 2011; Neumeister et al., 2008).

5-HT has been implicated in social modulation of startle responses in the African cichlid *Astalotilapia burtoni* (Whitaker et al., 2011). In this species, dominant males show increased startle probability and lower escape thresholds when compared to subordinate males (Neumeister et al., 2010), perhaps as a compensation of the increased conspicuity caused by brighter body coloration and higher activity (Medan and Preuss, 2014; Neumeister et al., 2010). The behavioral increases in startle are accompanied by increased excitability of M-cells to auditory stimuli, as well as a reduction on the inhibitory drive (Neumeister et al., 2010). The 5-HT_2 receptor antagonist ketanserin decreases feedback inhibition in subordinate, but not dominant, African cichlids (Whitaker et al., 2011). These represent presynaptic mechanisms, since only 5-HT_{5A} and 5-HT_6 receptors are expressed in Mauthner cells (Whitaker et al., 2011).

7. Conclusions

Different regions from the rostrocaudal axis appear to be involved in detecting, identifying, processing, and responding to aversive stimuli in fish. In general, threats are detected and processed at the level of the Dm, which may also be responsible for response selection at certain situations. This region is homologous to the mammalian frontotemporal amygalar cluster (Maximino et al., 2013a). The “classical model” of the role of the amygdala in fear involves aversive learning in the frontotemporal amygdala, while the behavioral output would be mediated by the autonomic/limbic amygdala (Vargas et al., 2012). Recent evidence, however, suggests that the frontotemporal amygdala is involved in encoding emotional events (including aversive stimuli and contexts) with reference to particular sensory features, while the autonomic amygdala encodes the motivational or af-

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fective significance (Balleine and Killcross, 2006). This is consistent with our hypothesis that the Dm also is responsible for mounting appropriate coping (active vs. passive) responses.

A parallel circuit for negative incentive and coping has been described in the habenula as well. The vHb appears to represent aversive expectation values and modulate aversive behavior via the median raphe nucleus (Amo et al., 2014). Similarly, a dHBM-vIPN-MRN appears to be involved in resilience to aversive stimuli and/or active coping, while the dHBL-iIPN-GC appears to be involved in selecting appropriate responses (Okamoto et al., 2011). So far, it is not known if this circuit is parallel to the (more classical) amygdalar/Dm one, or whether there are neuroanatomical connections between Hb nuclei and Dm and/or Vs, but an indirect projection to the vHb via the ventral entopeduncular nucleus has been described (Lal et al., 2018). Differently from the disinhibitory Dm-Vs circuit, however, the Dm-vEN-vHb circuit is excitatory. It is possible that both circuits run in parallel and compete (or collaborate) to decision-making in the GC.

The participation of these pathways needs to be interpreted in the context of a wider modulation of overall brain state, given the relationships between these circuits and downstream neuromodulators, such as dopamine and serotonin. In the case of the habenular circuit, this is more clear, since both the dHb and the vHb appear to produce their effects by modulating activity in the raphe; as a result, modifying serotonergic phasic and tonic responses in projections from the raphe. However, extensive serotonergic projections have been described to the telencephalon of zebrafish, with important projections to the Dm and Vs/Vp (Lillesaar et al., 2009). Therefore, it is highly likely that not only do neuromodulators such as monoamines act as mediators in the responses, but these transmitters also provide important feedback to the more rostral regions involved in threat detection and response selection (Figure 1B)

In addition to this “slow” pathways (the “forebrain circuit”), a “quick-and-dirty” pathway for visual aversive stimuli is organized in the optic tectum (the “midbrain circuit”)(Carr, 2015). This midbrain circuit is sensitive to stimulus size and critical angle, and switches from approach (small

stimuli probably mean “food”) to avoidance (large stimuli arriving at a specific angle probably mean “predator strike”). This circuit projects to motor regions of the medulla and spinal cord, as well as to the GC, initiating and/or modulating escape responses that are highly stereotypical. Moreover, a putative indirect OT-Dm projection, via the lateral preglomerular nucleus, has been proposed in fish as an analogue of the superior colliculus-pulvinar-amygdala pathway of mammals (Carr, 2015). It is expected that visually threatening stimuli activate the first circuit and bypass the Hb-IPN/raphe-GC or the Dm-Vs-GC circuits. Very little is known about the GC of zebrafish – contrary to the great importance that is given to the periaqueductal gray of mammals as a hub for defensive behavior (see Motta et al., 2017, for a recent review) –, an interesting research opportunity to clarify these circuits.

The forebrain circuit is functionally similar to LeDoux’s (1998, 2000) concept of a “high road”, providing slower but highly processed sensory information with affective tones, while the midbrain circuit would be functionally similar to the “low road”. In both the proposed forebrain circuit and the “high road”, threatening stimuli pass through increased processing of the nature of the sensory stimuli to reach a more precise identification; however, in LeDoux’s original formulation the high road refers to cortical processing of sensory information before reaching the amygdala, while the low road refers to information that is sent directly from the thalamus to the amygdala. Not only is the isocortex not the main target of sensory thalamic projections in zebrafish, the participation of putative homologues in aversive behavior has not been established (but see Aoki et al., 2013). Indeed, Carr (2015) suggested that a “high road” is absent in fish, which would only possess a “low road”. The present review, however, suggest that some granularity in this interpretation is necessary, given that a differentiation between responses mediated by the forebrain and midbrain circuits is possible.

The participation of the habenular pathways in neurovegetative responses is also less clear, but a amygdalar-hypothalamic circuit has been suggested on the basis of c-Fos and p-ERK activity

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(Faustino et al., 2017; Randlett et al., 2015). Aversive stimuli, including CAS and physical stressors, induce cortisol (Abreu et al., 2017b; Schirmer et al., 2013) and norepinephrine and epinephrine release (Maximino et al., 2014). This is consistent with a classical model of amygdalar control of fear in rodents, in which CEXA-hypothalamic projections regulate the neurovegetative responses to threats (Misslin, 2003). Based on coherence analysis of c-Fos expression to CAS (Faustino et al., 2017), it is suggested that the Dm activates inhibitory Vs neurons, disinhibiting downstream hypothalamic mechanisms.

What is the "switch" that regulates active vs. passive coping? What conditions favor switching from the forebrain to the midbrain circuits? While the neural bases of these changes are presently unknown, environmental characteristics which lead to decision-making have been described thoroughly. For example, threat probability and distance vary in a continuum that restricts attention (Andersen et al., 2016), and therefore whether fast or slower responses are needed (Brown et al., 1999; Fanselow and Lester, 1988; Kavaliers and Choleris, 2001; Laundré et al., 2010; McNaughton and Corr, 2004; Perusini and Fanselow, 2015). Thus, decision-making is biased towards escape (flight) or fight responses when the threat is proximal, while avoidance and freezing are elicited when threat is distal (Fanselow and Lester, 1988; McNaughton and Corr, 2004; Perusini and Fanselow, 2015). Similarly, the decision to freeze or flee is dependent on environmental affordances, such as the availability of escape routes (Blanchard and Blanchard, 1988). It has been suggested that habenular circuits act as comparators for these stimuli, biasing the organism towards careful approach or escape (Okamoto et al., 2011). An exciting possibility is that habenular circuits produce this effect by modulating the activity of serotonergic projections to the Dm and/or Vs and to the OT. This hypothesis is consistent with the Deakin/Graeff hypothesis for the role of serotonin in switching responses from escape towards risk assessment (Deakin and Graeff, 1991; Graeff et al., 1997; Maximino, 2012).

Much work is still needed to identify the regions that are responsible for aversive behavior in fish. The present review suggests some of the regions involved and presents a roadmap and framework for future research. The evolutionary relevance is clear, but it is also important to consider the implications of these findings for work in the field of behavioral models and experimental psychopathology: establishing homologies between regions involved in similar behavior strengthens the hypotheses that these behaviors are indeed conserved, an assumption of most models that is rarely tested (Maximino et al., 2010b) and of which depends the construct validity of these models. This implication opens up novel avenues for future research which ought to be prolific.

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