Habenu as the experience-dependent controlling switchboard of behavior and attention in social conflict and learning
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The habenula is among the evolutionarily most conserved parts of the brain and has been known for its role in the control of behavior to cope with aversive stimuli. Recent studies in zebrafish have revealed the novel roles of the two parallel neural pathways from the dorsal habenula to its target, the interpeduncular nucleus, in the control of behavioral choice whether to behave dominantly or submissively in the social conflict. They are modifiable depending on the internal state of the fish such as hunger and play another important role in orientation of attention whether to direct it internally to oneself or externally to others. These studies, therefore, are revealing a novel role for the habenula as the integrated switchboard for concordedly controlling behavior either as a winner with self-centered (idiothetic) attention or a loser with others-oriented (allothetic) attention.

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Introduction
The habenula (Hb) and its efferent projection termed the fasciculus retroflexus to the interpeduncular nucleus (IPN) are among the most conserved components of the brain throughout the vertebrate evolution [1]. In fact, they are found even in the jawless vertebrates such as hagfish (Mixinoidea) and lamprey (Petromyzontoida) [1–3]. In mammals, the Hb is a complex nucleus composed of lateral and medial subnuclei. The recent studies in mammals have put the central spotlight to the lateral habenula as the encoder of the aversive prediction error which plays an important role in the adaptive aversive reinforcement learning [4]. In contrast, relatively little is known about the roles of the medial habenula. Quite intriguingly, recent studies have been revealing its role for controlling behaviors in social conflict, based on the research carried out on its homolog in zebrafish [5]. Here, we review the recent progress in our understanding on the habenula as the switchboard for control of emotional and social behavior.

In zebrafish, the Hb can be divided into two subnuclei: the dorsal habenula (dHb) and ventral habenula (vHb) (Figure 1a). Judging from their efferent targets and the spatio-temporal transcriptome analysis at the single-cell level of the Hb both in zebrafish and mouse, the dHb and vHb correspond to the mammalian medial and lateral habenula, respectively [6–9] (Figure 1b). The dHb can be further divided into two different subnuclei: lateral subregion (dHbL) and medial subregion (dHbM). The dHbL project to the dorsal and intermediate part of the IPN (dIPN and iIPN, d/iIPN) and dHbM project to the ventral and intermediate part of the IPN (vIPN and iIPN, v/iIPN) [10]. The vHb directly send projection to the ventral-anterior corner of the median raphe (MR) [6,7]. The dHb is known to show conspicuous left-right asymmetry. In the left dHb, the dHbL is larger than the dHbM, while the dHbM is larger than the dHbL in the right dHb [10]. Because of this size asymmetry of the dHbL versus dHbM, the left dHb appears mostly project to the dIPN, while the right dHb appears mostly project to dIPN. (Regarding the mechanisms for the generation and its physiological significance of this asymmetry, please refer to Refs. [11–18]).

The axons from the dIPN project bilaterally to the dorsal directions, through the region putatively corresponding to the dorsal raphe (Figure 1a). They further extend laterally around the medial longitudinal fascicle, then turn caudally and elongate through the longitudinally extended region known as the griseum centrale (GC) or the dorsal tegmental area (DTA), which underlies the rhombencephalic ventricle, bilaterally forming a fasciculated fiber bundle [19] (Figure 1a). The DTA is the periventricular structure that most likely includes the regions corresponding to mammalian periaqueductal gray (PAG), dorsal tegmental nucleus and nucleus incertus. The IPN, the
The dorsal habenula (dHb)-interpeduncular nucleus (IPN) pathway, its evolutionary conservation and roles for aversive behavioral control. 
(a) Schematic illustration showing dorsal oblique view (left panel) and lateral view (right panel) of the zebrafish dHb-IPN pathways. OB: olfactory bulb; Tel: telencephalon; P: pineal organ; PP: parapineal organ; TeO: tectum opticum; Cbl: cerebellum; IL: inferior lobe of hypothalamus; MR: median raphe; DR: dorsal raphe; DTA: dorsal tegmentum area; MLF: medial longitudinal fascicle.
(b) Comparison between the zebrafish and mouse Hb subnuclear structure.
(c) The trajectories of the wild-type (left panel) and dHbL silenced (right panel) zebrafish after presentation of the conditioned stimulus (CS, red light).
(d) Schematic illustration of the apparatus for active avoidance test. US, unconditioned stimulus (electrical shock).

PAG and the nucleus incertus have been implicated in control of behaviors under fear or stress conditions, suggesting that the dHbL-d/iIPN-DTA pathway might contribute to the modulation of fear behaviors. In contrast, the vIPN has the reciprocal neural connection with the MR [19] (Figure 1a).

Recent development in the technology to genetically manipulate zebrafish genome and to qualitatively analyze the adult zebrafish behavior has enabled us to genetically dissect the roles of different subregions of the Hb by taking advantage of the relatively simple structure of the zebrafish Hb complex to reveal its pleiotropic roles.

The role of the dHb-IPN pathway in the experience-dependent modulation of fear behaviors
To examine the role of the dHbL, we created the transgenic fish expressing the tetanus toxin light chain in the dHbL neurons to prevent the release of synaptic vesicles and interrupt neural transmission, and subjected these adult transgenic fish to the cued fear-conditioning tasks by using electrical shock as unconditioned stimulus and illumination with red lamp as conditioned stimulus [19]. After the learning was established, the control sibling fish showed enhanced agitated or flight behaviors (conditioned stimulus-evoked increase in turning frequency) during the conditioned stimulus presentations. In contrast, most of the dHbL-silenced fish did not show a similar response to the conditioned stimulus, but instead showed persistent freezing (Figure 1c). Even during the training period, we observed the behavioral difference between the control and dHbL-silenced fish. After the first encounter with the unconditioned stimulus (electrical shock) paired with the conditioned stimulus (red lamp), both fish frequently showed freezing at the same level. Such responses generally waned in the control fish as they experienced the paired conditioned stimulus and
unconditioned stimulus repeatedly. In contrast, the freezing tendency of the dHbL-silenced fish became higher than that of the control fish during the second conditioning session. A similar persistence to freeze after repeated aversive stimuli was also observed in the larval zebrafish with the ablation of the dHbL (Supplementary Information in Ref. [19]) or with conversion of the left Hb to the right Hb by the mutation causing the right isomerization [20].

Both agitation and freezing are the responses innately induced by the aversive stimuli. By agitation, animals actively try to escape from the aversive stimuli. In contrast, by freezing, animals passively wait for the aversive stimuli to pass away. Our results suggested that the dHbL-d/iIPN pathway is essential for fish to make the behavioral transition in the experience-dependent manner from the passive coping (freezing) to the active coping (agitation) with the aversive stimuli.

The roles of the dHbL-d/iIPN and dHbM-i/viPN pathways as the winner and loser pathways in the regulation of social conflict

Because the PAG regulates fight, flight, and freezing behaviors in mammals [21], we wondered if the dHbL-d/iIPN-DTA pathway signals information critical for fighting and flight behaviors during aggressive social conflicts.

To address this question, we isolated adult male zebrafish for 24 hours and then put them together to fight in pairs. Dyadic male zebrafish fights proceed in a stereotypic manner, starting with each animal exhibiting display behaviors, followed by circling and biting attacks, and ending when one fish shows fleeing behavior indicating surrender [22] (Figure 2a). The winner and loser fish continue to keep the tendency to win and lose respectively, if they are put in the fight again with the naïve fish within 24 hours after the initial fight, showing that the winner and loser fish maintain altered brain states.

In fact, by calcium imaging of acute brain slices to visualize neural activity after electrical stimulation of the Hb, we found an intense potentiation of the dHbL-d/iIPN-DTA pathway in the winner fish [5]. In contrast, in the loser fish, we observed intense potentiation in the dHbM-v/iIPN-MR pathway (Figure 2b). Further, the dHbL-silenced fish showed a significantly higher tendency to lose fights. In contrast, the dHbM-silenced fish became more resistant to surrender and tended to win, demonstrating that the dHbL-d/iIPN-DTA pathway and the dHbM-i/viPN-MR pathway are respectively responsible for the behaviors characteristic to the winners and losers, respectively [5] (Figure 2b).

The mechanisms by which the dHbL-d/iIPN-DTA and dHbM-i/viPN-MR circuits facilitate winner and loser behaviors may be due to their projections to the DTA and MR, respectively. The reduction in transmission of the dHbL-d/iIPN pathway with an accompanying reduction in the activity propagation to the DTA that contains a putative homologous brain region to the mammalian PAG might switch fish behaviors from attacking to fleeing. On the other hand, the dHbM-i/viPN pathway may increase the tendency to lose through inhibitory outputs to repress the activity of the MR that was proposed to reduce anxiety- and depressive-like behaviors by increasing resilience to aversive stimuli. As the dHbL and dHbM antagonistically modulate the outcome of fights, it is possible that the interaction between these two regions determines the fighting results. Therefore, it is possible that the two dHb-iPN pathways act as a binary switchboard to affect fight outcomes.

Potentiation of the dHbL-d/iIPN pathway in hungry zebrafish by orexin-promoted biased alternative splicing of the AMPA receptor gene

Animals change their behavior in a hunger state, by increasing aggressive behavior, increasing foraging even in shock-associated areas to obtain food, and shifting behavior from avoidance to approach behavior. Although hunger is an important factor for determining animal behavior, the effects of hunger on social behaviors and the underlying mechanisms has remained unclear. We found that six day-starved fish increased resistance to surrender and tended to win fights with stronger potentiation of the dHbL-d/iIPN-DTA pathway even before experiencing fight [23] (Figure 2c).

Intriguingly, six day-starvation induced a prolongation of the activation time of AMPA-type glutamate receptor (AMPAR) in the dIPN region by biased expression of gria3b-flip, an alternative splicing variant of the AMPAR subunit (Figure 2d). Since the flip-type AMPAR receptor variant has a longer activation time than the flop-type variant [24,25], increase in the expression of the flip-type variant in the synapse results in the potentiation of the synaptic transmission from the dHbL termini to the dIPN. We further showed that starvation-induced increase in the orexin/hypocretin signaling from the hypothalamic neurons is responsible for this biased alternative splicing and the subsequent potentiation of the dHbL-d/iIPN-DTA pathway [23] (Figure 2c).

This result demonstrates that the internal state of fish such as hunger plasticly modulates the set point of balance in the internal antagonism between the winner and loser pathway from the dHb.

A possible role of the dHbL-d/iIPN pathway in the attention shift from others to self

Navigation behavior, such as foraging, is excellent to study how animals are engaged in attending to specific information to achieve favorable outcomes [26]. The
choice to use different navigation strategies, that is, whether to depend on landmarks or dead-reckoning, allows to conceptually divide information in terms of differences of the source. In landmark navigation, animals perceive the external cues through primary sensory modalities, such as olfaction, hearing, and vision. In contrast, dead-reckoning relies on an integrated internal signal such as vestibular and motor command signaling.

We designed an operant learning paradigm for obtaining appetitive reward (food) to monitor the learning and decision making process of zebrafish by adopting either the internal-directional information (left or right turn) or the external-cued information (toward the red-colored or blue-colored goal) with an automatic training system [27**] (Figure 3a,b). We found that the zebrafish can achieve the training paradigm by exploiting both internal-directional information and external-cued information (Figure 3c,d). By measuring the learning capacity with the different attended information with repeated rule shifts, we showed that zebrafish could retrieve associated memory while the rule was applied again.

Quite intriguingly, we further found that the dHbL-silenced or dHbL-ablated fish showed the specific defect in learning by exploiting the internal-directional information, while keeping its capacity to learn by using external-cued information unaffected (Figure 3e). This result demonstrated that the dHbL is indispensable for utilization of internal-directional information but not for external-cued information in the zebrafish operant learning behavior [27**].
Using a brain-wide functional screen, a recent study revealed that the IPN circuit may encode the left-right turning rate in zebrafish larvae under perceptual decision-making tasks [28**]. The authors showed that larvae zebrafish reacted to the left-ward or right-ward moving dots projected on a screen with varying directional coherence. Larval zebrafish used their observation of moving dots in the time scale of seconds to make judgments whether to flip the tail leftward or rightward with corresponding directional neural activities in the IPN. As the neurons in the habenula showed the ramping activity in response to the presented moving dots, the IPN may function as the processor for transforming integrated visual information into the motor output. Further, the authors predicted that this functional unit could be also engaged in other behavioral circumstances such as operant conditioning. Our study provides experimental evidence supporting that the dHbL-d/iIPN-DTA pathway indeed plays an essential role in operant conditioning learning by using directional information.

The recent research in mice has indicated that interbrain synchrony could reflect the social hierarchy, that is, neurons in the prefrontal cortex in winners respond better to their own behavior compared to the loser animals, while the losers’ neurons respond more to the opponent winners’ behavior [29**]. Considering that the dHbL-/d/iIPN-DTA pathway plays essential roles both in facilitating fish to behave as winners and in decision making depending on the internal-directional information (left or right), the winning experience in dyadic fighting could
synergistically induce a weight shift of information selection from other-directed to the self-centered selection, concomitantly with the brain state changing towards being more egocentric (Figure 4).

The essential role of the vHb in adaptive avoidance from the aversive cue and in the social conflict

In the classic fear conditioning, zebrafish were put in a confined tank to keep them inescapable from the unconditioned aversive stimulus (electrical shock) during the training period (Figure 1c). Therefore, the possible choice in the response to the conditioned stimulus was either of two innately encoded responses to the aversive stimuli, that is, agitation or freeze [19]. However, in different situations, that is, active avoidance learning (Figure 1d), where zebrafish were given more freedom in selecting their behaviors to adaptively avoid receiving the aversive stimulus, we found that the other part of the Hb, that is, vHb, plays a more essential role [7,30].

The transgenic fish with silencing of the vHb activity by expression of tetanus toxin showed a large reduction in the ratio of fish becoming successful learners in active avoidance compared to sibling control fish [7]. However, quite intriguingly, when we train the vHb-silenced fish for the classical fear conditioning, they exhibited a conditioned stimulus-evoked increase in turning frequency (agitation) as a learned response similar to control fish, indicating that the vHb is dispensable for acquiring innately programed response to the aversive conditioned stimulus in classical fear conditioning [7]. Kisspeptin1 is a neuropeptide specifically expressed in the vHb. The fish with the knockout of the kiss1 gene also show the similar phenotype [31,32].

When the fish with the silenced vHb were put in the fight with the wild-type fish, they mostly lost. However, these loser fish do not behave like the wild-type losers. The wild-type loser stays at the bottom corner of the tank quietly to avoid the further attack by the winner, but the vHb-silenced fish repeatedly swims back to the center of the tank where the winner fish is swimming, and continues to receive attacks by the winner. Namely, this fish cannot learn how to behave properly to avoid receiving the attack by the winner (see Supplementary Information and Movies in Ref. [5]).

In the social conflict of mice, the experience of attacking the submissive mouse is memorized as pleasure by the dominant mouse, because taking dominant status in social conflicts induces inhibition in the activity of the neurons in the lateral part of the lateral habenula which indirectly causes activation of the dopaminergic neurons in the ventral tegmental area (VTA) by way of the inhibitory neurons in the rostromedial tegmental nuclei (RMTg) [33,34]. Further, it was recently shown that activation of the orexin/hypocretin neurons causes inhibition of the neurons in the lateral habenula, and...
predispose mice to attacks [35]. Although the zebrafish vHb neurons are the homologs of the mice neurons in the most medial subregion of the lateral habenula in mice which has the direct projection to the MR [6,36–38], the neurons homologous to the mice neurons projecting to the RMTg have not been identified in the zebrafish habenula.

A loser of a single fight can recover from the loser state within one day. However, when fish were kept defeated for six consecutive days, they become unable to recover from the loser state for as long as a month [39]. The vHb in these defeated fish shows heightened activity [39]. Considering that the lateral habenula of the mice, the evolutionary homolog of the zebrafish vHb, is activated in the state of learned helplessness [40], this repeatedly defeated fish could also be a fish model of depression. Recently, the vHb was shown to be activated also in the larval fish after receiving inescapable shocks repeatedly, and to be responsible for the transition of the behavior from the active (tail flipping) to the passive (freezing) coping of the stress caused by the repeated shocks [41].

Conclusion

Both dHb and vHb are involved in experience-dependent modification of aversive responses after learning, but they contribute to this process in different manners. The dHb plays an important role in the selection of the behavior from the innately encoded repertoire such as (active) flight or (passive) freeze as the conditioned fear response, while the vHb is essential for adaptively constructing the best behavioral strategy in a goal-directed manner for the active escape from the aversive stimulus. Once the adaptive goal-directed behavior was acquired, the dHbL-silenced juvenile zebrafish was reported to show reduced adaptability in the reversal learning of the assigned value (safe or dangerous) to the compartments with different colors in passive avoidance learning, although this fish showed no defect in learning the correct behavior by the original rule, suggesting another role for dHbL in reversing the learned rules [42].

In the dyadic fights for social dominance or submission, the two pathways from the dHb to the IPN play antagonistic roles either to facilitate winning or losing, and the pathway from the vHb plays an essential role for the loser to adaptively learn appropriate behavior to avoid further attacks from the winner. These pathways are dynamically modified under the influence of the experience or the internal state of the fish such as hunger and contribute to the choice of the most suitable behaviors to cope with aversive stimuli and social conflicts. Further, the dHbL-d/iIPN pathway is involved in controlling internal (self-centered)/external (other-directed) selection of information, suggesting that the dHb/IPN pathway could act as the integrated switchboard for concurrently controlling zebrafish to behave either as a winner with self-centered (idiocentric) attention or a loser with others-oriented (allocentric) attention (Figure 4).

CRediT authorship contribution statement

Hitoshi Okamoto wrote the manuscript with the collaborators, Bor-Wei Chennig, Haruna Nakajo, Ming-Yi Chou and Masae Kinoshita who contributed greatly to accomplish the original works cited in this article.

Conflict of interest statement

Nothing declared.

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