Coping with Stress, One Habenula Neuron at a Time

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Animals often switch from active to passive coping behavior when exposed to inescapable stress. In a recent issue of *Cell*, Andalman et al. (2019) now demonstrate that this switch is mediated by gradual recruitment of activated habenula neurons in zebrafish.

When faced with a threat, animals typically try to either subdue it (“fight”) or escape it (“flight”). If successful, the chosen strategy was likely well worth the effort. However, what if these strategies fail? At some point, it might be better for the animal to cut its losses and wait for a better opportunity to escape. Indeed, many species show this pattern of behavior. The initial fight-or-flight behaviors are examples of “active coping,” while the latter, energy-conserving behavior (usually decreased mobility), is a form of “passive coping.”

Coping strategies have been studied not only out of ecological interest but also because of their relation to stress-related disorders, such as depression. More than 50 years ago, Overmier, Maier, and Seligman discovered that dogs given inescapable shock would later fail to escape the shock when presented the opportunity (Maier and Seligman, 2016). The dogs developed a passivity that resembled that of people with depressive disorders. The escape failure was called “learned helplessness” and spawned a field of research dedicated to understanding its psychological and biological underpinnings, as well as its relationship to mood disorders. Although passive coping may be adaptive during inescapable stress, the generalization of passivity to escapable situations is clearly not adaptive. In this way, passive behavior may be related to the pathological motivational impairments seen in depression.

About 10 years later, Porsolt, Le Pichon, and Jalfre described a new test of passive behavior—the forced swim test—in which rodents would initially try to escape when placed in water but eventually swim just enough to stay above the surface (Porsolt et al., 1977). The key finding was that rodents injected with various types of antidepressants, but not other compounds, showed less passivity in the forced swim test without an increase in locomotion (tested separately). Subsequent studies showed that prior stress—a major risk factor for depression—increased passive behavior in the forced swim test, further strengthening the link between passivity in the forced swim test and depression. However, concerns remain about how well passive behavior in the forced swim test (an apparent adaptive response to inescapable stress) models depression symptoms, as well as how closely the effects of antidepressants in this test mimic their therapeutic action. This is because antidepressants can immediately affect passive behavior in the forced swim test, but their therapeutic effects are usually not observed for weeks in people. Thus, although it is likely that some of the same brain processes underlying passive coping also contribute to depression, what these processes are and how they relate to depression are not clear.

With this in mind, Andalman et al. (2019) turned to zebrafish, whose size and transparency allow imaging of whole-brain activity in live animals. They first showed that inescapable shock causes freely swimming fish to initially increase their swimming speed, consistent with active coping, and then decrease their swimming speed below that of control, unshocked fish, consistent with passive coping (Figure 1A). Similar to studies in rodents (Garcia et al., 2008), the start of passive coping was delayed by treatment with ketamine, a rapidly acting antidepressant. Recovery of swimming speed depended on the context. If fish were put in the same context as that used for inescapable shock, they recovered slowly. However, recovery was faster if fish were put in contexts with different water and visual stimuli. In addition, adult fish could learn to escape the shock if given the opportunity. These experiments showed that slower swimming was not simply due to habituation to shock or damage to the fish.

To monitor whole-brain activity during inescapable shock, Andalman et al. (2019) first used light-field microscopy in head-fixed larvae and found that only the ventral habenula (vHb; homolog of the mammalian lateral habenula, LHb) increased activity after inescapable shock. This result fits with studies showing that a large proportion of neurons in the habenula is activated by aversive stimuli (Hikosaka, 2010). Using piezo-focus two-photon microscopy to resolve individual neurons, Andalman et al. (2019) then showed that average vHb activity rose steadily over the course of the shock protocol. Intriguingly, this was due to staggered recruitment of vHb neurons into the activated assembly (Figure 1B) rather than synchronous gradual increases in activity, an effect that was blocked by pre-treatment with ketamine. Nearby habenula neurons were recruited at similar latencies, indicating spatial coherence among an otherwise large degree of independent activity within the population. The average activity of the serotonergic raphe nuclei, the main output of the vHb (Amo et al., 2014), decreased during the shock protocol; however, unlike the vHb, individual raphe neurons did not change their activity in a staggered manner (Figure 1B). Re-exposure to shock produced faster decreases in the fishes’ tail movements and a larger increase in vHb activation and revealed a correlation between changes in movement and the vHb (but not the raphe) response.

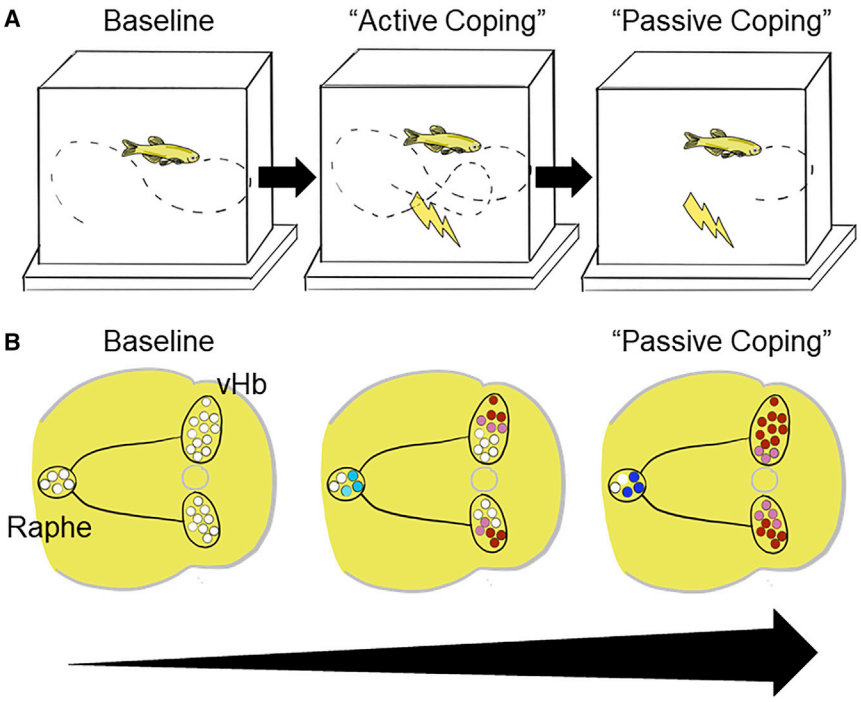

Figure 1. Behavior and Neuronal Activity during Inescapable Stress in Zebrafish
(A) Initial increase in swimming speed ("active coping") during shock followed by a decrease in swimming speed ("passive coping").
(B) Gradual recruitment of activated ventral habenula (vHb) neurons (red) and decrease in raphe activity (blue) during shock.

To further probe the link between vHb and raphe activity and passive behavior, Andalman et al. (2019) optogenetically stimulated vHb neurons and showed that this reduced swimming speed; inhibition of serotonergic neurons in the raphe produced the same effect. In contrast, inhibition of the vHb in shocked fish increased swimming speed. These results are consistent with a model wherein inescapable shock activates vHb neurons, causing inhibition of raphe neurons and passive behavior. Also consistent with this model, Andalman et al. (2019) showed that stimulation of the vHb inhibited the raphe (although at an 8–10 s delay). Brain-wide modeling of the observed activity patterns suggested changes in functional connectivity between the raphe and habenula and within the habenula itself during inescapable stress.

Many studies in mammals indicate an important role for the LHb in stress processing, passive behavior, and depression (Proulx et al., 2014). The emergence of the homologous vHb as a key region for stress responsivity in zebrafish suggests that this area of the habenula is part of a primal network for responding to threat. In addition, Andalman et al. (2019) now identify the precise pattern of activity within the habenula that occurs during passive behavior. Perhaps the most surprising discovery is that although a large proportion of vHb neurons were activated by the shock protocol, consistent with mammalian studies, they "turn on" with different latencies. These findings indicate that although there may be homogeneity in the coding properties of habenula neurons, there is heterogeneity in their response timing (at least in some conditions). Thus, the activity of habenula neurons is more independent of each other than previously appreciated. This independence might permit greater informational content within the habenula, greater robustness to aberrant input activity, and/or independent activation of downstream neural circuits.

The results of Andalman et al. (2019) also fit with another study showing important roles for the vHb and raphe in behavioral responses to threat (Amo et al., 2014). However, this study found that vHb activity is important for learning to escape shock (a form of active coping). How can vHb activity promote both active and passive coping behavior? One possibility is that vHb activity is necessary for generating negative reinforcement signals that drive escape learning in addition to its role in passive coping. Optogenetic manipulations of the vHb during escapable stress could test this possibility. It might also be important to know which cell types in the vHb are responsible for active and passive coping. Perhaps different cell types are involved in different responses to threat. Further studies are also needed to clarify the effects of vHb activity on raphe neurons; although Andalman et al. (2019) found that vHb stimulation caused delayed inhibition of the raphe *in vivo*, Amo et al. (2014) found that vHb stimulation excited raphe neurons in brain slices.

In mammals, the LHb controls the activity of not only the serotonergic raphe nuclei but also midbrain dopamine neurons (Proulx et al., 2014). Primate LHb neurons encode similar reward computations as midbrain dopamine neurons (Hikosaka, 2010). Although many aspects of habenula connectivity and function are conserved between zebrafish and mammals (Amo et al., 2014), zebrafish do not have a midbrain dopamine system. Going forward, it will be interesting to see whether mammals show a similar recruitment of habenula neuron activity during inescapable stress and, if so, whether the relevant cell types are the same ones that process reward.

Notably, the recruitment of habenula activity during inescapable stress observed by Andalman et al. (2019) was prevented by ketamine treatment, providing a link to depression. Along these lines, it would also be important to know whether the habenula responses seen in Andalman et al. (2019) are specific to *inescapable* stress. Escapable stress is believed to be more benign than inescapable stress, which has been linked to depression and post-traumatic stress disorder (Maier and Seligman, 2016). Therefore, if escapable stress recruits less habenula activity than inescapable stress, habenula hyperactivity may be a biomarker of stress-induced motivational pathology. Alternatively, controllability of stress might only be manifest in the activity of targets of the habenula, such as the raphe and midbrain dopamine

systems (Dolzani et al., 2016; Tye et al., 2013; Warden et al., 2012).

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