Cortisol's impact on Locomotion during Zebrafish Novel Tank Testing and a Shorter Evaluation of Human Analogs looking towards the use of Virtual Environments in Neuroendocrine Research.

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There is currently an acceleration of behavioral neuroendocrinology research interest exploring covarying levels of the stress hormone cortisol and how it alters motility patterns in zebrafish. This field growth is likely due to this paradigm's versatility. The main idea of this work is that cortisol levels rise in response to the phenomenological stress present in an organism's internal and external environment, which corresponds to distinct behavior pattern alterations that present to researchers as motility changes. Thus, this review will first briefly compare the cortisol axes of zebrafish and humans. Then explore a portion of the recent literature assessing the correlation between circulating cortisol and anxiolytic locomotion, focusing primarily on cortisol's covariation to zebrafish motility. Within this portion, there will be several topics evaluated, the impacts of perceivable environmental conditions, the mixed results of using net chasing (NC) as a stressor, and the usefulness of using psychoactive drugs or conspecific alarm substances (CAS) to inform how these may impact the human stress response. Finally, this review will conclude with a brief evaluation of cortisols' effects on human motility in the real and virtual worlds while assessing the usefulness of virtual environments (VEs) for studying human neuroendocrine responses in the future.

In the simplest sense, cortisol is an end product of either the human hypothalamic-pituitary-adrenal (HPA) axis or the zebrafish hypothalamic-pituitary-interrenal (HPI) axis (Thau et al., 2021; Quadros et al., 2021). When met with cognitive stress, both organisms first releases corticotropin-releasing hormone (CRH) from the hypothalamus, which signals the downstream surge of adrenocorticotropic-releasing hormone (ACTH) from the anterior pituitary gland. This rise of ACTH initiates the production and secretion of cortisol, a glucocorticoid hormone of the steroid class, which then acts as a negative inhibitor of

the two upstream tropic peptide hormones. In humans, cortisol is produced in and secreted from the middle cell layer of the kidney's outer tissue layer, known as the zona fasciculata of the adrenal cortex. In contrast, in zebrafish, the origin is the interrenal tissue, a combination of chromaffin and intermixed steroidogenic cells (Tsalafouta et al., 2014). Although there are structural differences between these organisms, the pathway is roughly the same. Research indicates that cortisol regulates many of the body's physiological responses to the phenomenological experience of stress/anxiety/risk avoidance by binding to receptors in nearly every organ tissue, like the respiratory, cardiovascular, skeletomuscular, and others (Thau et al., 2021). This phenomenological stressor can be either internal or external and is not dependent on subjective valance. Although this specific hormone is not entirely conversed throughout evolutionary history, research finds that the HPA/HPI axes are a similar active component in these two organisms' general stress response (Ottaviani and Franceschi, 1996).

Given the high-conversed nature of this pathway, the manipulability of the parameters of their environment, and their short life spans, this model organism offers researchers a fast, repeatable, and reliable way to experiment with stress responses that can potentially be extrapolated backward to humans (Cachat et al., 2010). The legitimacy of these experiments is due to the two sides to the evaluative coin implemented during these studies. On the one hand, researchers perform anxiety-related behavioral assays where they observe physical responses, such as latency to move into a safe area of a tank, time spent at the top or bottom of a tank, and proclivity for being in a dark area versus a light one. All of which, when compared between populations, indicate whether the organism is subjectively stressed or not. On the other hand, there is the use of

endocrine-phenotyping assays, including high-throughput neurophenotyping and genetic mutation screenings, to determine what internal factors correlate with the displayed external behaviors. Altogether, combining a stressor with the behavioral output and interindividual variety to observe the range of impact stressors can have on locomotive behavior.

Zebrafish Motility

While there are many ways to delineate this type of research, one area currently being explored is how the parameters of a zebrafish's perceivable surroundings impact their behavior and cortisol levels. Two aspects of this overarching idea in recent literature are the fish's housing environment and their shoal. A study by de Abreu et al. examines how housing tank lid color impacts whole-body cortisol and motility patterns in a novel tank test (2020). Their results are, fish housed in tanks with black or blue lids are less anxious than those housed in yellow or white lids. During this experiment, the first two groups show increased mobility, less thigmotaxis, and less whole-body cortisol. A second study by a different group of researchers evaluated another perceivable environmental condition: being in a shoal versus being alone during net chasing impacted stress (Giacomini et al., 2015). Surprisingly, their results show that fish, when chased in the shoal condition, had significantly higher whole-body cortisol levels than their solo counterparts, a finding the research team attributes to chemical and behavioral cues of the fish's shoal mates. These studies together inform future researchers of two things. These organisms do not exist in a vacuum, making standardization of whole life conditions/environments a critical concern of future research, and that there might be other unvalidated aspects of these experiments that are producing confounding results.

One of these unsettled aspects of this literature appearing to produce split results is net chasing as a stressor. This particular predictable chronic stress (PCS) type is the staple stressor in many zebrafish studies examined for this literature review (De Abreu et al., 2014; Giacomini et al., 2015; Ramsay et al., 2009; Shams et al., 2017). However, two very recent studies suggest that PCS of this type might be one of these aspects assumed to produce stress but does not impart stress to the expected magnitude or maybe at all in some cases. The most damning evidence comes from a study that focuses primarily on how acute net stress impacts both anxiety-related behavior and whole-body cortisol (Aponte and Petrunich-Rutherford, 2019). This team examined the behavior and whole-body cortisol levels of zebrafish that were netted and held above water for thirty seconds. They found no differences between anxiety-like behavior in either a novel tank test or light/dark preference test and no statistically significant differences in whole-cortisol between controls and netted individuals.

Similarly, another study, which is currently at the stage of being a journal pre-proof, examining the effects of NC and CAS found that NC for two minutes twice a day over fourteen days also did not increase whole-body cortisol levels but did mildly affect light/dark test latency (Quadros et al., 2021). They noted a habituation effect. These disparate results likely indicate that differences in how the research team performs these stressors impact the impartment of stress onto the zebrafish. Collectively, suggesting the need for further research and technique standardization within the stressor portion of this research.

Anytime a researcher is physically interacting with an experiment, there is room for error, but some stressors implemented in these anxiety-related motility studies do not

require researcher manipulation that could differ from one experiment to another. One such stressor type is the discharge of psychoactive soluble drugs or CAS, which permits researchers to evaluate stress responses in these fish using drug dosage, which is less fallible than NC and provides pharmacologically translatable knowledge to humans. Many studies use this model of drug introduction to evaluate how the release of these chemicals increases and decreases the animal's stress response.

One such study by Tang et al. evaluates the development and neurotoxicity effects of environmentally dispatched antidepressant venlafaxine on the zebrafish larvae stress locomotion and cortisol levels, also showing that administration of melatonin alleviates these symptoms (2021). Their team assessed one, ten, and one hundred micrograms per liter, finding that while one hundred micrograms per liter cause sluggish behavior, while one microgram per liter has the opposite effect increasing motility during both light and dark cycles, but especially in dark conditions. This activity corresponded to not only cortisol increases but the increased expression of "cortisol-regulated genes," being the Star, Cyp11a1, and Cyp11b1. Not only does this particular drug study inform researchers about the possible effects of this antidepressant in humans, but as the articles state, "environmental levels of venlafaxine are reported as high as 2.19 μg/L in wastewater effluent from a site in Minnesota." Studies like this will provide drug regulators the ammo needed to establish stricter control over these substances as they likely have unintended ecological effects.

A second psychoactive drug study by Cachat et al. focuses less on the impact of a singular psychoactive drug and its ecological and behavioral impacts, instead choosing to examine an extensive range of psychoactive drugs and their corresponding

behavioral/cortisol changes in a comparative fashion and sets up a strict protocol for future research (2010). The range of chemical stressors used in this study was: ethanol, fluoxetine, morphine, caffeine, acute alarm pheromone, and the team included a high anxiety leopard strain as another control. The teams' results indicate each of these chemicals has some varying effect on both cortisol and observed motility, but warns that some drugs, such as hallucinogens, may cause an increase in one that does not correlate to increases in the other. However, the most important takeaway from this article, in particular, is their rigorous development of a novel tank testing protocol, which is an issue noted early in this review. The group lists fifty steps and timing estimates for some that provide standardization necessary for this type of research.

Human Motility

At this point, with the evidential establishment of a correlation between cortisol and zebrafish motility patterns, this review will transition to similar cortisol-related motility patterns in humans, which presently relies more heavily on physiological measurements rather than endocrine sampling. One such study paving the way was a pair of experiments by Walz et al., who used a global positioning system and an open-field test paradigm to demonstrate that high-anxiety individuals present with statistically significant thigmotaxis during city market walks over their healthy control counterparts (2016). The group's first experiment assessed agoraphobics versus healthy controls, while the second looked at high versus low anxiety participants, and while both groups show thigmotaxis, the agoraphobics did so to a greater degree. However, it is essential to note that this study's anxiety state discernment was done via trained clinician diagnoses but did not include any endocrine samples. While an issue for the context of this review paper, other

evidence suggests urinary and salivary cortisol is higher in people with panic and anxiety disorders (Kathol et al., 1988; Vreeburg et al., 2021). Therefore, it is rational to assume that cortisol fluctuations play a similar correlative role in human motility differences between these two phenotypes.

Interestingly, this motility evidence alone shows that, like with zebrafish, the phenomenological experience of anxiety likely alters human motility patterns. However, unlike zebrafish, the feasibility of creating highly manipulable large-scale open field tests for human endocrine studies has ethical and technical limitations (Parsons, 2015). With the coevolution of virtual reality (VR) technologies, the feasibility, but not the ethical nature, of creating an ecologically informed and psychologically controlled laboratory experiment to study the endocrine response of human anxiety from an ethological point of view is becoming a reality. While currently, much of the psychological VR literature surrounds increasing these technology's performance to make them more marketable to consumers, this press for system optimization has had the unintended consequence of opening a new avenue to human behavioral research. The researchers interested in observing human's behavior from an ethological standpoint can now do it through the proven lens of motility patterns and the corresponding physiological measures.

One such study by Rodrigues et al. does precisely this by evaluating participants' movement patterns of participants in three VR scenarios (2020). This experiment used a within-subject design, as scenarios one and two were mildly arousing environments designed to collect individual movement patterns that the team used to predict cardiovascular arousal, such as heart rate variability and heart rate, in the third high arousal "dark maze" scenario. While again, this study does not include endocrine

sampling, cardiovascular arousal is a byproduct of parasympathetic activation, linked to increases in cortisol secretion. Therefore, their results show that novelty exploration difference predicts interindividual physiological stress response differences and aligns with the notion that VR motility patterns, like real-world motility patterns, correspond to endocrine variation.

Considering the literature of this review in totality, this author will propose a possible future direction. While the novelty of VR is enamoring many researchers, video games are also VEs. These games are more ecologically valid as people play them regardless of any study and are much more challenging than any current VR game. Thus, the recording of similar lines inside these even more ecologically valid and immersive environments might serve as a proxy for authentic human behavior and endocrine response to stressful situations. Horror games like Phasmophobia or realistic first-person shooters like Escape from Tarkov, which are considered highly stressful within the gaming community, might serve as objective diagnostic tools for anxiety or panic disorders in the future. However, first, a validation process must occur, showing that motility patterns differ between healthy controls and anxiolytic participants and that these results strongly covary with both subjective anxiety measures and physiological/endocrine samplings. To achieve this validation, it will be imperative that researchers consider the range of data coming out of the animal studies, such as aspects of the perceivable environment, stressor type validation, and if drug treatments impose stress alterations during gameplay.

This hypothetical direction aside, in both zebrafish models and human studies, motility differences correlate with stress responses. The literature surrounding zebrafish

as a model organism is far from settled, as there are still uninformed aspects, like housing and test tank conditions, and pitfalls, such as unvalidated stressors, that pose an issue for those trying to pull truth from these studies. However, some of this literature, specifically the studies examining soluble drugs and alert chemicals, produces solid and translatable findings to human neuroendocrine research. These behavioral assays also inform human behavioral-endocrine analysis tools on top of these robust findings from the drug trials. As VR and VE research intermesh with behavioral, cognitive, and endocrine science, these tools may be the future of human behavioral research as they allow the animal paradigms to translate to human participants. However, if the validation process and pitfalls do not inform these studies, VE usage will likely be unfruitful.

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