Comparing behavioral responses across multiple assays of stress and anxiety in zebrafish (*Danio rerio*)

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Accepted 5 September 2012

Abstract

Animals encounter stressful situations multiple times throughout their lives and often successfully cope with them. Individuals vary in the nature and intensity of their behavioral and physiological response to stressors, often representing correlated and qualitatively distinct coping styles (e.g., proactive and reactive). These alternative coping styles are ways an animal can overcome a variety of stressful situations, which ultimately can have important fitness consequences. Here we use zebrafish (*Danio rerio*) recently wild-derived and selectively bred for amount of stationary behavior (High and Low lines) and a classic domesticated strain (AB) to document the utility of these zebrafish strains in understanding coping mechanisms. The Low Stationary Behavior (LSB) line of zebrafish displayed significantly lower stress and anxiety-related behaviors than the High Stationary Behavior (HSB) across six stress and anxiety-related behavioral assays. In some assays, we observed strain differences in behavior within three minutes of the start of the trial. Males also showed reduced levels of anxiety-related behaviors relative to females in two assays. Comparing wild-derived and domesticated strains, the AB line displayed significantly lower levels of anxiety-related behavior in half of the assays. This study demonstrates that our selectively bred lines from wild-caught zebrafish (HSB, LSB) exhibit consistent and divergent behavioral stress responses across multiple distinct assays. Hence these lines may prove useful in understanding the proximate and ultimate mechanisms of coping with stress and anxiety.

Keywords

stress, anxiety, coping style, *Danio.*
1. Introduction

Animals experience stressors in a wide range of natural contexts including resource defense and acquisition, mate search, and exploring novel environments. These natural stressors often involve an internal conflict between alternative options. Upon encountering novel environments, stress and anxiety-related behaviors can be the result of conflict between exploring the area for potential mates, food resources, or territories with those of predation and injury. While individuals in a species vary in the response to stressful situations, how they overcome and cope with these stressors has been the focus of many studies (for reviews, see Cryan & Holmes, 2005; Overli et al., 2007; Koolhaas et al., 2010). Coping styles represent ways that an organism handles challenges and divergent coping styles may be maintained in a population due to differences in optimal behavioral phenotypes across variable environments (Wilson, 1998; Koolhaas et al., 1999; Dingemanse et al., 2004; Sih et al., 2004). A coping style is a suite of behavioral and physiological responses to stress that are typically stable across time and context with alternate styles characterizing different groups of individuals (Koolhaas et al., 1999, 2010).

Individuals with a proactive coping style are generally characterized by responding to novel environments/stimuli through (1) a feed-forward process (i.e., recalling previously engrained responses), where they actively move about and explore, and (2) having lower behavioral flexibility and physiological stress response (Koolhaas et al., 2010). On the other hand those exhibiting the qualitatively different reactive coping style will spend more time waiting for additional environmental cues before responding (e.g., high stationary behavior or ‘freezing’). Correlated with this sit-and-wait approach, these individuals also display higher behavioral flexibility and physiological stress response (Koolhaas et al., 2010). Although terminology describing qualitatively alternative responses (e.g., coping style, behavioral syndromes) may differ based on taxon, the existence of distinct coping styles is apparent in teleosts, reptiles, birds, and mammals (Sih et al., 2004; Koolhaas et al., 2007; Overli et al., 2007; Brelin et al., 2008).

To understand the natural proximate and ultimate mechanisms that can explain alternative coping styles, it is useful to utilize animals that are not far removed from their wild counterparts. Many generations of selective breeding or domestication have been repeatedly documented to reduce variation