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Developing zebrafish models relevant to PTSD and other trauma- and stressor-related disorders

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ABSTRACT

While post-traumatic stress disorder (PTSD) and other trauma- and stress-related disorders (TSRDs) represent a serious societal and public health concern, their pathogenesis is largely unknown. Given the clinical complexity of TSRD development and susceptibility, greater investigation into candidate biomarkers and specific genetic pathways implicated in both risk and resilience to trauma becomes critical. In line with this, numerous animal models have been extensively used to better understand the pathogenic mechanisms of PTSD and related TSRD. Here, we discuss the rapidly increasing potential of zebrafish as models of these disorders, and how their use may aid researchers in uncovering novel treatments and therapies in this field.

1. Introduction: clinical PTSD

Post-traumatic stress disorder (PTSD) and other trauma- and stress-related disorders (TSRDs, Table 1) are severely debilitating stress-indiced neuropsychiatric illnesses characterized by dysregulation in the processing of stimuli associated with trauma. TSRDs are primarily defined by three clusters of symptoms: re-experiencing trauma, robust avoidance behavior and hyperarousal (Chavez, 2006; Heinzelmann and Gill, 2013; Qureshi et al., 2011). PTSD affects approximately 1–8% of the general population, and represents a serious societal and public health concern (Chavez, 2006; Kessler et al., 1995). Exposure to a severe stressor or trauma, such as violence or combat stress, is a common trigger of PTSD and other TSRDs (Alisic et al., 2014; Pietrzak et al., 2014) (also see (Kang et al., 2003)). While not all impacted individuals develop PTSD, susceptible individuals demonstrate a set of clear symptoms (Table 1) currently recognized as PTSD (Chavez, 2006; Pietrzak et al., 2014; Warner et al., 2013).

In addition to clinical PTSD and other TSRDs, affected patients often show high comorbidity with various other neuropsychiatric disorders, such as depression, anxiety, drug abuse and psychoses (Fig. 1) (Anderson et al., 2014; Rojas et al., 2014). Vulnerability to PTSD also demonstrates a high heterogeneity, as only 18–36% of trauma-exposed individuals develop PTSD (Heinzelmann and Gill, 2013), and its symptoms are expressed in a sex-specific manner (Jin et al., 2014; Shvil et al., 2014). However, the pathogenesis underlying the vulnerability and development of PTSD and other TSRDs is largely unknown (Sherin and Nemeroff, 2011), and merits further translational analyses.

Mounting evidence suggests that biological dysregulation of the neuromediator (e.g., opioid-, glutamate-, noradren-, seroton- and oxytocine-ergic) pathways and neuroendocrine cascades is involved in the pathophysiology of PTSD and TSRD (Hageman et al., 2001). In addition to environmental triggers, clinical PTSD also has genetic risk factors (Brewin et al., 2000; Domschke, 2012). For example, the genes catechol-O-methyltransferase (*COMT*), tryptophan hydroxylase (*TPH*) *1*, *TPH2*, and serotonin transporter (*SERT*) have been associated with higher risks of developing PTSD in various clinical cohorts (Goenjian et al., 2012; Kolassa et al., 2010; Valente et al., 2011). Epigenetic influences have also been suggested to mediate PTSD vulnerability in genetically susceptible individuals (Koenen et al., 2011; Ressler et al., 2011; Rusiecki et al., 2012; Smith et al., 2011; Uddin et al., 2010, 2011). Thus, given the high complexity driving the disorder's development and susceptibility, greater investigation into candidate biomarkers and specific genetic pathways implicated in both risk and resilience to trauma becomes critical (Heinzelmann and Gill, 2013).

2. Animal models of PTSD

Recognizing the growing clinical importance of PTSD and related TSRD, various experimental animal models have been developed to

Abbreviations: BDNF, brain-derived neurotrophic factor; COMT, catechol-O-methyltransferase; CRF, corticotropin releasing factor; HTS, high-throughput screens; HPA, hypothalamic–pituitary–adrenal; HPI, hypothalamic–pituitary–interrenal; LSD, lysergic acid diethylamide; MDMA, 3,4-methylenedioxymethamphetamine; MAOIs, monoamine oxidase inhibitors; NPY, neuropeptide Y; OT, oxytocin; PTSD, post-traumatic stress disorder; PP1, protein phosphate-1; SSRIs, selective serotonin reuptake inhibitors; SERT, serotonin transporter; SAA, serum amyloid A; TPH, tryptophan hydroxylase; TSRDs, trauma- and stress-related disorders; TCAs, tricyclic antidepressants.

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target different aspects of PTSD pathogenesis (Table 2, Fig. 2). This area of research is rapidly developing, and more new experimental genetic and pharmacological animal models of PTSD continue to emerge (Caramillo et al., 2014; Daskalakis et al., 2013; Goswami et al., 2013; Matar et al., 2013; Ursano et al., 2007). Clearly, every animal model has multiple limitations (McGonigle and Ruggeri, 2014; Stewart and Kalueff, in press), and all these PTSD models have various degrees of construct, face and predictive validity (Goswami et al., 2013; Matar et al., 2013). Traditionally, the field strongly relied on rodent (and, to a lesser extent, primate) models of PTSD, most of which have intuitively been based on acute or prolonged exposure to stressors (Table 2, Fig. 2) — the same factor that triggers clinical TSRD (Alisic et al., 2014; Pietrzak et al., 2014). Therefore, the availability of experimental paradigms mimicking PTSD pathogenesis (Table 2) and the possibility of developing sensitive in-vivo screens for anti-PTSD medication (see further) are encouraging. (See Fig. 3.)

Numerous animal models have been extensively used to better understand the pathogenic mechanisms of PTSD (Ursano et al., 2007). For example, many rodent models of PTSD commonly employ stressors to instill fear, reduce social interaction, and induce cognitive impairments that are implicated in behavioral abnormalities associated with stress-related disorders. These models include several fear conditioning models (e.g., the electric foot shock), adaptations of stress/restress paradigms, as well as genetically manipulated and psychosocial models, such as maternal separation and exposure to predators or aggressors (Adamec and Shallow, 1993; Caramillo et al., 2014; Cohen et al., 2004; Garrick et al., 2001; Harvey et al., 2006).

Fear conditioned methods are widely used in rodents to assess molecular and genetic factors underlying stress-related behaviors (Zovkic and Sweatt, 2013). The exposure to the uncontrollable electric foot shock in rodents conditions fear by amplifying the rodent's sense of helplessness, a common facet to human depression (Dunn and Swiergiel, 2008). Recapitulating clinical PTSD symptoms, rodents (even in the absence of foot shocks) continue to exhibit deleterious effects, such as an increase of acoustic startle response when provoked with contextual reminders (Rasmussen et al., 2008). Prognostic indicators, such as the acoustic startle response, help to subdivide rodents into categories of those predisposed to developing stress-like disorders and those more resilient. Importantly, several newly identified candidate genes are involved in these fear responses, suggesting that behavioral differences are due to predisposed genetic variation. For example, in the absence of *stathmin* gene, rodents reveal a "fearless" phenotype, even following foot shocks (Martel et al., 2012). High levels of stathmin protein are found in the amygdala of rodents with elevated anxiety-like behavior, whereas manipulations in stathmin transcript levels can significantly alter behavioral anxiety. Additionally, the corticotropin-releasing factor 1 receptor antagonist, SSR125543, albeit not involved with the hypothalamic–pituitary–adrenal (HPA) axis, equally diminishes anxiety-like response following a shock exposure (Parker et al., 2012). The opposite is observed in the absence of gastrin-related peptide receptor genes, which invokes a more timid quality in rodents (Martel et al., 2012). Moreover, various inbred mouse strains display different stress behaviors, some of which elevate or suppress startle response. For example, the SWR/J mouse strain appears as a promising model to study stress resilience (Szklarczyk et al., 2012), whereas the BALB/c strain displays increased sensitivity to stressors (Brinks et al., 2007), collectively suggesting that stress resiliency and sensitivity (highly relevant to PTSD) involves a complex set of genetic–phenotypic interplay in rodents.

Several rodent and primate models of maternal separation or loss are used to mimic early adversity (Monk et al., 2012), another clinically relevant PTSD-related phenotype. For example, rhesus monkeys of early social isolation showed characteristics of aggressive play and irregular reproductive activity (Feng et al., 2011). Early maternal separation in primate models often resulted in long-term alterations in neuroendocrine response to stress, such as augmented levels of cortisol and a pronounced frontal lobe area with more dependence to the right hemisphere (Davenport et al., 2008). This asymmetric activity of the frontal lobes is consistent to patterns found in human infants with developing anxiety personalities (Avram et al., 2010; Moscovitch et al., 2011).

Overall, various mammalian models of PTSD have been recently comprehensively evaluated in the literature (Caramillo et al., 2014; Daskalakis et al., 2013; Goswami et al., 2013; Matar et al., 2013; Ursano et al., 2007), and will not be discussed here in-depth. However, the importance of cross-species disease modeling has recently been recognized in biological psychiatry and translational neuroscience research (Kas et al., 2007; Kas et al., 2011; Stewart and Kalueff, in press). For example, critical and biologically meaningful information about shared and evolutionarily conserved 'core' disordered circuits and molecular pathways can only be obtained if several model organisms are used in integrative disease modeling (Kalueff and Stewart, 2014; Stewart and Kalueff, in press). From this point of view, among various other species, the zebrafish (*Danio rerio*) becomes a particularly interesting organism for modeling complex brain disorders (Kalueff et al., in press-a) and their relation to stress (Kalueff et al., 2014a, 2014c; Stewart et al., 2014a).

3. Zebrafish models relevant to PTSD

3.1. General rationale

Can zebrafish be used to develop translational models relevant to PTSD? Importantly, zebrafish have recently emerged as a promising model to study complex neuropsychiatric illnesses (Kalueff et al., 2014a, 2014c; Stewart et al., 2014a). They possess an extensive array of quantifiable behavioral phenotypes, many of which are bi-directionally sensitive to a wide range of stress-inducing and anti-stress drugs, generally paralleling rodent and clinical data for these agents (Kalueff et al., in press-b). Moreover, zebrafish possess *evolutionarily conserved* neural mechanisms and mediator systems with high homology to rodents and humans, as the same set of genes often regulates critical aspects of stress responses in humans and in 'lower' species (Kalueff et al., in press-b; Kaslin and Panula, 2001). Zebrafish exhibit high genetic homology with humans as well, with 70–80% of shared nucleotide sequence (Dooley and Zon, 2000). Perhaps even more importantly, the amino acid sequence of proteins (with 60–90% sequence homology) and especially their functionally relevant catalytic or ligand-binding domains (approaching 100% sequence homology) are highly similar between zebrafish and humans (Renier et al., 2007). At the practical level, zebrafish are easy to manipulate genetically, very cost-efficient, easy to breed, and can be housed in large numbers in relatively small space, rendering them an ideal species for high-throughput genetic and pharmacological screening (Ali et al., 2012; Kokel and Peterson, 2008, 2011; Laggner et al., 2012). The relative simplicity of zebrafish (vs. rodents) CNS organization, although seemingly a limitation of the model, can also be used advantageously — for example, enabling a better isolation of shared, ancient 'core' biological variables and molecular pathways associated with PTSD and other stress-related disorders (Kalueff et al., 2014a). Collectively, this suggests that zebrafish possess all major qualities necessary for an animal model of PTSD (see (Caramillo et al., 2014) for a recent review).

3.2. Behavioral models

Zebrafish display a rich repertoire of complex and overt behavioral responses to a wide variety of stimuli (including those inducing anxiety or fear), which closely parallels that of mammals (Blaser et al., 2010; Cachat et al., 2010c; Jesuthasan, in press; Stewart et al., 2012a). Because abnormal human stress responses, such as fear underlying PTSD, are linked to the malfunction of neurobiological mechanisms (i.e., neural circuitry and molecular pathways) that originally evolved to subserve the avoidance of predators or other harmful agents in nature, the

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