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Individual differences in conditioned fear expression are associated with enduring differences in endogenous Fibroblast Growth Factor-2 and hippocampal-mediated memory performance

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ABSTRACT

Rodent studies of individual differences in fear expression following Pavlovian fear conditioning are thought to provide useful means by which to examine the factors associated with vulnerability and resilience to anxiety and trauma- and stressor-related disorders in humans. We have recently demonstrated that rats that naturally exhibit low levels of conditioned fear have greater hippocampal expression of the neurotrophic factor Fibroblast Growth Factor-2 (FGF2), relative to rats that naturally exhibit high levels of conditioned fear. In the present study we determined whether individual variance in conditioned fear expression is associated with distinct behavioral profiles across a range of tasks designed to assess expression of trait anxiety and non-emotional memory performance, and whether the differences in hippocampal FGF2 are relatively stable across time. Results indicated that, relative to rats naturally exhibiting low levels of fear, rats naturally exhibiting high levels of fear in the presence of a previously conditioned cue and context also showed heightened levels of trait anxiety, reduced ability to discriminate between a previously conditioned context and a safe context, and impaired performance on the hippocampal-mediated place recognition task, but not on the non-hippocampal-mediated object recognition task. Moreover, differences in hippocampal FGF2 expression were evident between high and low fear rats even three months following the tests for conditioned fear expression. Together, these results suggest that individual differences in conditioned fear expression may be mediated partly by enduring differences in hippocampal functioning.

1. Introduction

While many people are exposed to traumatic events at some time in their lives, only a small proportion subsequently develops anxiety or trauma- and stressor-related disorders, like Post Traumatic Stress Disorder (PTSD) (Yehuda & LeDoux, 2007). Identifying factors associated with individual differences in response to trauma may provide a greater understanding of the mechanisms underlying vulnerability and resilience to trauma-related psychopathology. One approach used to address this issue has been to examine individual differences in the expression of learned fear in rodents. Learned fear is typically studied via Pavlovian conditioning, whereby a previously neutral conditioned stimulus (CS) is paired with an innately fearful unconditioned stimulus (US; such as a footshock), until the CS acquires the capacity to elicit fear in the absence of the US. In some cases the CS is a discrete cue, such as a tone or light, whereas in other cases the CS comprises the diffuse contextual cues present when the US occurs (Phillips & LeDoux, 1992). Fear conditioning is directly relevant to the etiology of trauma- and stressor-related disorders like PTSD, which develops following exposure to a traumatic event, leading to heightened fear of discrete cues (e.g. sights, sounds, smells) and contextual cues (e.g. location, time of day) associated with that event (Difede, Olden, & Cukor, 2014; Foa, Steketee, & Rothbaum, 1989; Mineka & Zinbarg, 2006).

Just as humans exhibit substantial individual differences in response to trauma, research has established that rodents also exhibit significant individual differences in the expression of cued and contextual fear (Bush, Sotres-Bayon, & LeDoux, 2007; Duvarci, Bauer, & Paré, 2009). Recent investigations have explored the potential neurobiological correlates of individual differences in learned fear with the aim of identifying specific variables that may foster vulnerability and resilience following traumatic experiences. For example, we recently identified an association between individual differences in contextual fear and hippocampal expression of the neurotrophic factor Fibroblast growth Factor 2 (FGF2;

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Graham & Richardson, 2016). Rats that displayed lower levels of contextual fear had significantly greater hippocampal FGF2 protein, relative to rats that displayed higher levels of contextual fear. An identical pattern of results was obtained in a separate cohort of rats tested for cued fear. Combined with previous findings that an acute systemic administration of FGF2 reduces fear in the presence of an aversive CS (Graham & Richardson, 2009b), this suggests that FGF2 may function as an endogenous regulator of individual differences in learned fear.

Our recent findings are consistent with other studies that have demonstrated a negative correlation between trait anxiety (i.e., unlearned fear) and FGF2. For example, Perez, Clinton, Turner, Watson, and Akil (2009) selectively bred rats to exhibit high or low trait anxiety and found that highly anxious rats had significantly lower hippocampal FGF2 mRNA than rats with low trait anxiety. They further demonstrated that environmental enrichment reduced trait anxiety in highly anxious rats, an effect that was associated with an increase in hippocampal FGF2 mRNA. A reduction in trait anxiety was also observed following either chronic administration of FGF2 during adulthood (Perez et al., 2009) or a single injection of FGF2 on the first day of life (Turner, Watson, & Akil, 2012). In contrast, an increase in trait anxiety was observed in outbred rats following the selective knockdown of FGF2 mRNA (Eren-Koçak, Turner, Watson, & Akil, 2011).

Taken together, these findings suggest that in addition to regulating individual differences in learned fear, FGF2 may also be an endogenous regulator of trait anxiety. Such findings lead to intriguing hypotheses regarding FGF2's potential role in conferring resilience against the development of anxiety and stressor-related disorders. However, very little is known about the behavioral features of the high and low fear phenotypes following conditioning. For example, while FGF2 has been associated with expression of both contextual and cued fear in separate cohorts of rats, it is unknown whether individual differences in conditioned fear expression are similar across these different modalities of fear conditioning (i.e., cues and contexts). Likewise, while FGF2 has been associated with individual differences in both learned and unlearned fear expression in separate studies, it is unclear whether rats that display high cued and contextual fear also display high levels of trait anxiety (i.e., perhaps mediated by endogenous FGF2). While some studies have reported a link between learned fear expression and trait anxiety (Borta, Wöhr, & Schwarting, 2006; Duvarci et al., 2009), others have failed to find such a relationship (Bush et al., 2007). Moreover, the ways in which these high and low fear phenotypes may differ behaviorally beyond their performance in learned and unlearned fear protocols has rarely been explored. For example, given that learned fear expression depends on the capacity to acquire, consolidate, and recall a conditioning episode, it is plausible that individual differences in learned fear expression may reflect differences in learning and memory capacity more generally. Indeed one study has reported a positive correlation between individual differences in fear conditioning and performance across a range of learning and memory tasks in mice (Matzel et al., 2003). Finally, little is known about the stability of the underlying neurobiology of high and low fear phenotypes. For example, while we have reported that rats with low levels of conditioned fear exhibit high levels of hippocampal FGF2 two hours after being tested for fear expression (Graham & Richardson, 2016), it is unclear whether or not this is a transient stress response, or an enduring neurobiological characteristic. Each of these various issues were examined in the present study. Developing a more robust profile of high and low fear phenotypes is essential to facilitate our understanding of their functional relevance, face validity, and clinical utility.

In the present study, we subjected a large cohort of rats to a mild conditioning event, involving a single pairing of a white-noise CS with a low-intensity footshock in a distinct context. Rats were tested for both cued- and context-elicited fear, 24 h apart, to determine the concordance of high and low fear phenotypes when the fear is elicited by discrete cues or the context. Rats that exhibited a consistent phenotype (i.e., high or low fear to both the context and the cue) were subsequently tested on a number of tasks to determine the relationship between conditioned fear expression, trait anxiety, and (non-fearful) hippocampal-dependent and -independent learning and memory performance. Finally, neural levels of FGF2 were quantified three months after fear conditioning to determine the stability of the relationship between hippocampal FGF2 and conditioned fear expression over time.

2. Method

2.1. Subjects

Experimentally naïve adult male Sprague–Dawley rats (280–430 g, N = 32), aged 8–10 weeks at the commencement of experimentation, were housed in groups of 8 at the UNSW School of Psychology, Australia. Rats were maintained on a 12 h light–dark cycle (lights on at 0700) and food and water were available *ad libitum*. All procedures were approved by the UNSW Animal Care and Ethics Committee and were carried out in accordance with The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (8th edition).

2.2. Apparatus

2.2.1. Fear conditioning

Two sets of two identical experimental chambers (24 cm long × 30 cm wide × 21 cm high) were used for fear conditioning and test procedures. All four chambers were housed in separate wooden cabinets to minimize external auditory and visual stimulation, and ventilation fans provided low, constant background noise. An infrared video camera mounted on the rear wall of the cabinets recorded the behavior of each rat inside the chamber. The chambers were wiped clean with tap water after each use.

The two sets of chambers differed in a number of visual and tactile features, and served as distinct contexts for experimental procedures. Both the fear conditioning procedures and the test for contextual fear expression were done in chambers designated as Context A. The front walls, rear walls, and ceilings of these chambers were constructed of clear Perspex. The sidewalls were made of stainless steel, in one of which a high-frequency speaker was embedded. The floor consisted of stainless steel rods set 1.5 cm apart, and connected to a shock generator. These chambers were illuminated by infrared light from the video camera.

Test for cued fear expression was conducted in chambers designated as Context B. These chambers differed from Context A in that only the rear walls were constructed of clear Perspex. The front walls were covered with a piece of patterned paper (2.5 cm wide vertical black and white stripes), and the ceilings were overlaid with a sheet of opaque Perspex. The stainless steel rod floors were covered with a sheet of opaque Perspex. White light from a table lamp illuminated these chambers.

The CS was a 10 s white noise (4 dB above background) and the US was a scrambled foot-shock (1 s, 0.6 mA).

2.2.2. Elevated Plus Maze (EPM)

The EPM consisted of a wooden plus-shaped platform elevated 50 cm above the floor, with two open arms and two closed arms (each 50 cm long × 12 cm wide), and an open square in the center (10 × 10 cm). The closed arms were surrounded by wooden walls