Altered fear memory and neurotransmitter regulation in a mouse model of trait anxiety

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Introduction

In terms of evolution, fear learning represents an advantageous response mechanism to potentially threatening situations. Yet, in different forms of anxiety disorders such as post-traumatic stress disorders or phobias fear learning is altered in terms of over-consolidation and the extinction of the learned fear is impaired in the course of psychotherapy. The mechanisms, why some people develop experience-based anxiety while others don't despite the same aversive experiences, are not entirely clear, but genetic factors are thought to determine the propensity to develop an anxiety disorder. Here, we investigated the relationship between a genetic predisposition to heightened trait anxiety and experience-based learned fear in a psychopathological mouse model and subsequently explored underlying neurobiological mechanisms in the amygdala which is known to be critically implicated in the acquisition, storage and expression of conditioned fear [1]. For that purpose, individuals with either high (HAB) or normal (NAB) anxiety-related behavior derived from the outbred CD-1 mouse strain following a selective breeding approach [2,3] were subjected to classical fear conditioning paradigms consisting of fear acquisition, fear expression and fear extinction sessions.

Methods

Experiments were carried out on adult HAB and NAB mice bred for their innate level of anxiety-related behavior at the Department of Pharmacology and Toxicology, University of Innsbruck, Austria (for details see [2,3]). Animals were fear conditioned by pairing the context or tone cue (80 dB of white noise or 9 kHz sine wave) with a mild electric foot shock and subjected to fear extinction training by repeated presentation of the conditioned stimulus only [4,5] while microdialysis samples were being collected. Microdialysis probes (CMA, Sweden) were chronically implanted into the right amygdala of mice (1.6 mm caudal, 2.9 mm lateral, 4.2 mm ventral to bregma). Concentrations of catecholamines in microdialysates were radioenzymatically determined according to previous protocols [6]. In addition, the induction of the immediate early genes c-Fos and Zif268 and the activity of various proteinkinases was used to map neuronal activation in the amygdala of HAB and NAB mice using western blotting and immunohistochemistry, respectively [5,7].
Results and Discussion

During conditioning both mouse lines showed increased fear responses to either the tone or context as assessed by freezing behavior. 24 h later HAB mice displayed more pronounced conditioned responses to the conditioned stimulus as compared with NAB mice. Since NAB mice show normal strain-specific fear expression, it is proposed that trait anxiety determines stronger fear memories in the HAB line and/or a weaker ability to inhibit fear responses, which has been also described in humans [8]. Accordingly, HAB mice required more extinction trials than NAB animals in order to reach basal freezing levels and showed spontaneous recovery of extinguished conditioned fear over longer periods of time supporting the idea that the strength of fear memory may be correlated with the resistance to fear extinction. Thus, HAB mice reflect patients that suffer from any anxiety-related disorder and display deficits in extinction-based exposure therapy. Following fear conditioning we observed an increased phosphorylation of the protein kinase B (AKT) and an increased induction of the two immediate early genes c-Fos and Zif268 in the (baso-)lateral amygdala of HAB as compared with NAB mice. This finding points towards a critical involvement of the amygdala in the acquisition and storage of heightened learned fear in highly anxious individuals. The increased activation of the (baso-)lateral amygdala coincided with an excessive and prolonged release of amygdaloid noradrenaline during and after fear conditioning suggesting that over-stimulation of the noradrenergic system may enhance fear memory formation in HAB mice. In contrast, on the next day when fear extinction training was performed, noradrenaline levels did not differ between the two lines while dopamine release was reduced in the amygdala of HAB as compared with NAB mice. Taken together, HAB mice represent a powerful model for the functional analysis of neuroanatomical networks and neurobiological mechanisms underlying trait anxiety-related enhanced susceptibility to learned emotionality and recovery of fear after successful extinction.

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References