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# Susceptibility to stress in transgenic mice overexpressing TrkC, a model of panic disorder

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ABSTRACT

Stressful life events increase the susceptibility for subsequent onset of psychiatric disorders in humans. Previous research has implicated neurotrophins in the onset of some stress-related diseases, such as major depression disorder, post-traumatic stress disorder or panic disorder. We have tested the hypothesis that the neurotrophin-3 (NT-3)/TrkC system is a genetic interface mediating the deleterious effects of stress on the initiation of panic disorder and other pathologies. To this aim, we have analyzed the functionality of HPA axis and the behavioral consequences of different types of stressful conditions in a mouse model of panic disorder, which overexpresses TrkC, the high affinity-receptor for NT-3 (TgNTRK3). Our results reveal that TgNTRK3 mice exhibit an altered circadian corticosterone rhythm that is reversed by clonidine treatment, but normal expression of genes involved in the control of the hypothalamus–pituitary–adrenal (HPA) axis (CRH, GR) and normal corticosterone response to acute and chronic stressors. In contrast, they exhibit an altered pattern of activation of stress-related brain areas and showed enhanced anxiety-related behavior and more passive strategies than wild types under some chronic stress conditions. We conclude that TgNTRK3 mice present differences in their response to stress characterized by subtle changes in the HPA axis, marked changes in acute stress-induced brain activation and altered coping strategies, suggesting a key role of TrkC receptor in the stress neural circuitry and in the behavioral consequences of chronic stress.



## 1. Introduction

Stress susceptibility in humans is a major risk factor to develop neuropsychiatric disorders, and a contributing factor to post-traumatic stress disorder (PTSD), major depressive disorder (MDD) or panic disorder (PAND) (Rubin, 1989; Schreiber et al., 1996; Yehuda, 2002). In addition to an inability to develop adequate coping strategies (Goldstein et al., 1987; Stokes, 1995), these stress-related disorders are often accompanied by alterations of the central and peripheral components of the hypothalamus–pituitary–adrenal (HPA) axis. However, such alterations differ among the three pathologies. Thus, PTSD and MDD appears to be characterized by higher brain corticotrophin-releasing hormone (CRH) activity, but peripheral HPA hormones, particularly cortisol, are elevated in MDD, whereas hypocortisolism has been observed in some PTSD populations (Belmaker and Agam, 2008; de Kloet et al., 2006; Kellner and Yehuda, 1999; Stam, 2007). In contrast, PAND is characterized by normal brain CRH activity, and normal or high plasma cortisol levels (Abelson et al., 2007; Kellner and Yehuda, 1999).

Recently, molecules involved in the development and plasticity of the central nervous system such as neurotrophins, have been suggested to play a role as etiological or predisposing factors in stress-related disorders (Duman and Monteggia, 2006; Gratacós et al., 2007; Govindarajan et al., 2006). In humans, linkage studies have revealed that single nucleotide polymorphisms in the neurotrophin-3 (NT-3) receptor, the neurotrophin tyrosine kinase receptor 3 (*NTRK3*) gene, encoding for TrkC, may be involved in the pathophysiology of PAND (Armengol et al., 2002) and obsessive–compulsive disorder (OCD) (Alonso et al., 2008; Mercader et al., 2008). In addition, genome scan studies (Holmans et al., 2004; McGuffin et al., 2005) showed a high evidence of linkage of the 15q25.3–26.2 region, that contains *NTRK3* gene, with recurrent MDD of early-onset, confirmed by linkage disequilibrium mapping of this region (Feng et al., 2008; Verma et al., 2008). These

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results suggest that *NTRK3* may have a function in the pathophysiology of stress-related disorders, probably by mediating or potentiating the effect of negative or stressful life events. Moreover, the expression of NT-3 and TrkC is ubiquitously distributed throughout the brain, but their mRNAs have been predominantly detected in the hippocampus (Lamballe et al., 1994), a region that plays a modulatory role in the HPA axis functionality and *in situ* hybridization studies have demonstrated TrkC expression in regions involved in the initiation and modulation of the stress response, such as the paraventricular nucleus of the hypothalamus (PVN) and other hypothalamic nuclei, at both prenatal and postnatal stages (Hassink et al., 1999).

It has been hypothesized that the alteration of neurotrophin expression driven by stress could be involved in the onset and pathophysiology of stress-related disorders, since they play an important role in translating neuronal activity into biochemical and structural plasticity (Lindholm et al., 1994). In fact, exposure to stress alters neurotrophin expression in brain (Pizarro et al., 2004; Smith et al., 1995a,b,c). Specifically, intense stressful stimuli, such as acute or chronic immobilization, reduce BDNF (Adlard et al., 2004; Murakami et al., 2005; Smith et al., 1995c) and increase NT-3 mRNA levels in the hippocampus (Smith et al., 1995c). In addition, early maternal separation followed by two acute exposures to stress in adolescence and adulthood, has been reported to increase NT-3 levels in the dorsal hippocampus (Faure et al., 2006) and glucocorticoids regulate neurotrophin mRNA levels in the cerebral cortex and hippocampus (Barbany and Persson, 1992; Hansson et al., 2000; Pizarro et al., 2004; Smith et al., 1995a,b,c). Regarding neurotrophin receptors, TrkB expression is also affected by glucocorticoids (Roskoden et al., 2004; Schaaf et al., 1998) and stress (Ueyama et al., 1997), and early postnatal corticosterone increases TrkC mRNA levels in the rat hippocampus (Roskoden et al., 2004).

Previous studies in our laboratory have validated for PAND TgNTRK3 mice, a transgenic mouse model that overexpresses TrkC. These mice show mild anxiety-like behavior in some tests, increased panic reaction and higher sensitivity to anxiolytics (Dierssen et al., 2006). Besides we detected an increase in locus coeruleus (LC) noradrenergic (NAergic) neurons (Dierssen et al., 2006), increased cellularity in the amygdala and hippocampus (Sahun et al., 2007a), and a differential neuronal activation pattern to anxiogenic drugs (Sahun et al., 2007b). Finally, electrophysiological studies revealed an increase in basal firing rate in the LC (Gallego et al., submitted for publication), and increased paired pulse facilitation and long term potentiation (LTP) in the hippocampus (Sahun et al., 2007a), which may influence for certain aspects of stress-related information processing.

We here propose that the dysregulation of NT-3/TrkC, which we previously demonstrated to be involved in PAND pathophysiology (Dierssen et al., 2006; Sahun et al., 2007a), could be important for the altered adaptation or sensitization to chronic stress in mood and anxiety-related disorders. To explore this possibility we have used TgNTRK3 mice (Dierssen et al., 2006), exploring the responses of these mice to different stressor intensities at the hormone, gene expression, brain activation and behavioral levels.

## 2. Methods and materials

### 2.1. Animals

In order to exclude possible positional effects we included in the analysis two different lines of TgNTRK3, with insertion of the transgene in different chromosomes. Same sex littermates were group-housed (2–4 animals per cage) in standard macrolon cages ($40 \times 25 \times 20$ cm) under a 12 h light/dark schedule (lights on at 07:00) in controlled environmental conditions of humidity (50–70%) and temperature (22 ± 2 °C) with food and water supplied *ad libitum*. We used adult (3–7 months of age) TgNTRK3 and wild type male littermates from at least four different litters, to reduce the impact of mothering or litter size effects. All animal procedures were approved by the local ethical committee (CEEA-PRBB), and met the guidelines of the Spanish (Decree 214/97 law 32/2007, and Catalan law 5/1995) and European regulations (EU directive n° 86/609, EU decree 2001-486) and the Standards for Use of Laboratory Animals n° A5388-01 (NIH). The CRG is authorized to work with genetically modified organisms (A/ES/05/I-13 and A/ES/05/14) and AAA is accredited for laboratory animal experimentation by the local government (Generalitat de Catalunya).

### 2.2. Blood sampling and biochemical analysis

Blood sampling for the determination of hormone levels in basal and stress conditions was performed by tail-nick procedure, wrapping the animal with a cloth and performing a 2 mm incision at the end of the tail artery. Massaging the tail allowed the collection of 100 µl of blood in an ice-cold EDTA-coated capillary tube (Sarstedst, Germany). Blood samples were kept on ice, centrifuged (15 min, 1600 g at 4 °C) and plasma transferred to clean, 2 mL microcentrifuge tubes. Plasma samples were stored at −20 °C until determination of corticosterone.

Corticosterone RIA used 125I-carboximethyloxime-tyrosine-methyl ester (ICN-Biolink 2000, Spain), synthetic corticosterone (Sigma, Spain) as standard, and an antibody raised in rabbits against corticosterone–carboximethyloxime–BSA kindly provided by Dr. G. Makara (Inst. Exp. Med., Budapest, Hungary). Plasma corticosteroid-binding globulin (CBG) was inactivated by low pH. All samples to be statistically compared were run in the same assay to avoid inter-assay variability. The intra-assay coefficient of variation was 4.9%.

### 2.3. Experimental design

In order to characterize the possible involvement of TrkC in PAND stress susceptibility, we analyzed TgNTRK3 mice under basal or stress conditions. Three experiments where conducted in which mice were exposed to stress. In the first, levels of corticosterone and brain activation pattern were evaluated after acute stress. In the second, levels of corticosterone were assessed in a single housing and chronic immobilization stress (CIS) paradigm. The last experiment assessed the behavioral and CRH/glucocorticoid receptor (GR) mRNA expression levels in a chronic environmental stress (CES) paradigm.

### 2.4. Circadian corticosterone rhythm

Male mice of either genotype (14 TgNTRK3 and 10 wild type) were used to analyze corticosterone circadian rhythm. Blood samples were obtained at 08:00 h (at the beginning of diurnal phase) and 20:00 h (at the beginning of nocturnal phase) (Moore and Eichler, 1976). A separate group of animals (12 TgNTRK3 and 14 wild type mice) was added to obtain basal corticosterone levels at 14:00 (diurnal phase) (Fig. 1).

To test the influence of the NAergic system on circadian corticosterone rhythm in TgNTRK3, mice were administered clonidine in basal conditions. Clonidine (Sigma, USA) was dissolved in saline solution (0.9%) and administered intraperitoneally (injection volume 10 mL/kg at 0.1 mg/kg). Fourteen TgNTRK3 and 14 wild type littermates distributed in four groups were treated with clonidine (8 TgNTRK3 and 8 wild type mice) or saline (6 TgNTRK3 and 6 wild type mice) 60 min before blood sampling at 14:00.