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Differential gene expression in a rat model of depression based on persistent differences in exploratory activity

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Abstract

Affective disorders are often accompanied by changes in motivation and anxiety. We investigated the genome-wide gene expression patterns in an animal model of depression that separates Wistar rats belonging into clusters of persistently high anxiety/low motivation to explore and low anxiety/high motivation to explore (low explorers and high explorers, LE and HE, respectively), in three brain regions previously implicated in mood disorders (raphe, hippocampus and the frontal cortex). Several serotonin-, GABA-, and glutamatergic genes were differentially expressed in LE- and HE-rats. The analysis of Gene Ontology biological process terms associated with the differentially regulated genes identified a significant overrepresentation of genes involved in the neuron development, morphogenesis, and differentiation; the most enriched pathways from the Kyoto Encyclopedia of Genes and Genomes were the Wnt signalling, MAPK signalling, long-term potentiation, and long-term depression pathways. These findings corroborate some expression data from other models of depression, and suggest additional targets.

1. Introduction

In recent years, animals bred or preselected for individual variability are proving to be increasingly useful in modelling aspects of human psychopathology, particularly anxiety-related disorders and addiction (Piazza et al., 1989; Landgraf and Wigger, 2002; Kabbaj, 2006; White et al., 2007; Pawlak et al., 2008). Differences in behavioural traits are accompanied by differences in monoaminergic neurotransmission and sensitivity to pharmacological manipulations (Piazza et al., 1989; Hooks et al., 1992; Rouge-Pont et al., 1998).

Behaviour of animals in unfamiliar environments is always a function of their natural curiosity and fear of the unknown

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(Harro, 1993); alterations in these motivational processes—low motivation and high anxiety—are regarded as core features of depression. The exploration box test that measures novelty-related behaviour in rats typically yields a distribution that strongly deviates from normality and facilitates separation of rats into groups of high motivation to explore/low neophobia and low motivation to explore/high neophobia (HE and LE, respectively), with relatively few animals occupying the middle ground. These differences in spontaneous exploratory activity levels are stable over time, persist with repeated testing and predict activity in other behavioural tests (Mällo et al., 2007). Compared to HE-animals, LE-rats are less active and more anxious in the elevated plus-maze and display passive coping strategies in the forced swimming test. In the fear conditioning test, LE-animals acquire a more persistent association between neutral and stressful stimuli (Mällo et al., 2007). The LE-rats also develop behavioural sensitization to repeated amphetamine treatment more readily (Alttoa et al., 2007). The HE- and LE-animals spend a similar amount of time in active social interaction, thus do not differ in their social anxiety (Mällo et al., 2007). The differences in novelty-related behaviour in HE- and LE-rats cannot be explained by the variations in general locomotor activity, as in the elevated plus-maze the number of entries into the closed arm of the maze is similar in HE- and LE-rats, and there are no differences in their activity in a novel home-cage like environment (Mällo et al., 2007).

The distinct behavioural profiles of HE- and LE-rats are also reflected in the differences in neurochemistry. We have shown previously that the HE-animals have higher basal and stimulated extracellular dopamine levels in the striatum but not in the nucleus accumbens (Mällo et al., 2007), and a higher proportion of dopamine-D_2 receptors in the functional high-affinity state (Alttoa et al., 2009). There are also differences between high and low exploring groups in the properties of the serotonergic system in the prefrontal cortex and hippocampus (Mällo et al., 2008) that may underlie their anxiety-related behaviours. Specifically, although serotonin release in the prefrontal cortex and dentate gyrus in baseline conditions is similar in HE- and LE-rats, the number of serotonin transporter binding sites in the prefrontal cortex is higher in the LE-animals. Thus, after blocking the serotonin transporters in the prefrontal cortex by a local infusion of citalopram, the serotonin release in that brain region is significantly increased in the LE group (Mällo et al., 2008). The drug-free, amphetamine-stimulated and amphetamine-sensitized behaviour of the HE- and LE-rats is differentially regulated by noradrenaline, as revealed after a partial noradrenergic denervation of locus coeruleus projections with a selective neurotoxin DSP-4 (Alttoa et al., 2005, 2007). Furthermore, the two groups have distinct cerebral metabolic activity in areas that are involved in defensive behaviours and cognitive processing of sensory stimuli (Matrov et al., 2007). Thus, the LE-rats demonstrate a behavioural profile that is characterized by low motivation to explore, high anxiety, high vulnerability to stress and cognitive rigidity—the core symptoms of depression, and thus, resembles a 'depressed' phenotype. Differences in monoaminergic neurotransmission between LE- and HE-rats are also compatible with the notion of LE-rats being an animal model of depression.

In order to investigate the genetic differences between the 'healthy' and the 'depressed', and identify novel mechanisms that contribute to or underlie the observed phenotype, genome-wide microarrays are the method of choice. Thus, the aim of the current study was to identify the genes and molecular pathways that contribute to the differences between HE- and LE-rats by examining the gene expression profiles in three brain regions most consistently implicated in the pathogenesis of depression and anxiety (the raphe, the hippocampus and the frontal cortex) using Illumina RatRef-12 BeadChips.

2. Experimental procedures

2.1. Animals

Male Wistar rats weighing 275–472 g (Scanbur BK AB, Sweden) were housed four per cage in standard polypropylene cages in a light controlled room (12-h light/dark cycle; lights on at 7:00 a.m.) maintained at 22 °C. Food and water were available *ad libitum*. All behavioural experiments were carried out between 13:00 and 19:00. All experiments were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by the Ethics Committee of the University of Tartu. All efforts were made to minimize the number of animals used and their suffering.

2.2. Apparatus

The exploration box test was conducted as described previously (Harro et al., 1995 and Otter et al., 1997). The exploration box was made of metal and consisted of a 0.5×1 m open area (side walls 40 cm) with a 20×20×20 cm small compartment attached to one of the shorter sides of the open area. The open area was divided into eight squares of equal size. In the open area, four objects, three novel and one familiar (a glass jar, a cardboard box, a wooden handle and a food pellet) were situated in certain places (which remained the same throughout the experiment). The small compartment had its floor covered with wood shavings and was directly linked to the open area through an opening (size 20×20 cm). The apparatus was cleaned with a dampened cloth after each animal. The exploration test was initiated by placing a rat into the small compartment, which was then covered with a lid for the time of the test. A single test session lasted 15 min and the following behavioural parameters were registered: (1) latency (of entering open area with all four paws), (2) number of entries into the open area, (3) time spent exploring on the open area, (4) line crossings, (5) rearings, and (6) number of unfamiliar object investigations. To provide an index of exploration the scores of line crossing, rearing and object investigation were summed for each animal and thus (7) the sum of exploratory events obtained.

2.3. General procedure

One hundred and sixty male Wistar rats were initially tested on two consecutive days for their spontaneous exploratory activity in the exploration box. The animals were classified as high or low explorers (HE or LE, respectively) based on the sum of the exploratory events on the second exposure to the exploration box test. Only animals that did not emerge from the small chamber ($n=49$) were categorized as low explorers, while rats belonging to the upper quartile of the number of exploratory events on the second exposure ($n=40$) were classified as high explorers. Of these animals, 12 HE and 12 LE-animals were randomly chosen for the microarray experiments. The rats were decapitated 10 days after the last behavioural experiment, their brains removed and the raphe nuclei, hippocampi and frontal cortex dissected on ice. The brain tissues were immediately frozen in liquid nitrogen and stored at −80 °C until RNA extraction.