Imaging trait anxiety in high anxiety F344 rats: Focus on the dorsomedial prefrontal cortex

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Abstract

Functional magnetic resonance imaging (fMRI) has become an important method in clinical psychiatry research whereas there are still only few comparable preclinical investigations. Herein, we report that fMRI in rats can provide key information regarding brain areas underlying anxiety behavior. Perfusion as surrogate for neuronal activity was measured by means of arterial spin labeling-based fMRI in various brain areas of high anxiety F344 rats and control Sprague–Dawley rats. In one of these areas, the dorsomedial prefrontal cortex (dmPFC), c-Fos labeling was compared between these two strains with immunolabeling. The effects of a neurotoxic ibotenic acid lesion of the dmPFC in F344 rats were examined in a social approach–avoidance anxiety procedure and fMRI. Regional brain activity of high anxiety F344 rats was different in selective cortical and subcortical areas as compared to that of low anxiety Sprague–Dawley rats; the largest difference (i.e. hyperactivity) was measured in the dmPFC. Independently, c-Fos labeling confirmed that F344 rats show increased dmPFC activity. The functional role was confirmed by neurotoxic lesion of the dmPFC that reversed the high anxiety-like behavior and partially normalized the brain activity pattern of F344 rats. The current findings may have translational value as increased activity is reported in an equivalent cortical area in patients with social anxiety, suggesting that pharmacological or functional inhibition of activity in this brain area should be explored to alleviate social anxiety in patients.
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1. Introduction

Multiple epidemiological studies have shown that genetics play an important role in anxiety disorders in humans (Hettema et al., 2001; Leonardo and Hen, 2006). Using

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inbred mice, this influence was confirmed by identifying several genetic factors that are associated with emotion-related behaviors in various procedures that model anxiety (Clement et al., 2002; Muigg et al., 2009; O'Mahony et al., 2010). In rats, genetic approaches for studies on anxiety have been limited and only few studies have compared outbred or inbred rat strains. One of the more robust findings is that inbred Fischer 344 (F344) rats display an anxious phenotype in several anxiety-based paradigms, such as the elevated plus maze, the black−white box and the social interaction test (Bert et al., 2002; Berton et al., 1997; Ramos et al., 1997; Rex et al., 1999), and may be a valid starting point to identify the neuronal pathways underlying high anxiety behavior.

Brain regions of greatest interest in the pathology of anxiety disorders, including social anxiety, are the amygdala, the anterior cingulate cortex, the ventromedial prefrontal cortex (vmPFC), the orbitofrontal cortex, the insula and the temporal areas (Amir et al., 2005; Canteras et al., 2010; Etkin and Wager, 2007; Goldin et al., 2009b; Phan et al., 2005; Shah et al., 2009; Stein et al., 2007). Multiple preclinical studies using lesions or pharmacological challenges were able to show the involvement of some of these areas in anxiety (Navarro et al., 2004; Rudebeck et al., 2007; Sajdyk et al., 1999; Sullivan and Gratton, 2002) but the findings are heterogeneous. A potentially valuable approach to better investigate the role of brain areas in high anxiety animals is the use of functional magnetic resonance imaging (fMRI) technology. It allows an indirect measurement of brain activity in many different areas at the same time in a non-invasive manner, with translational value. In contrast to the clinical setting, only few preclinical fMRI studies have examined the neuronal pathways that may be involved in anxiety-like behavior (Ferris et al., 2008; Kalisch et al., 2004; Nephew et al., 2009).

The main goals in the present study were: 1) to compare the behavior of F344 rats with that of a standard rat strain (Sprague−Dawley, SD) in the social approach−avoidance (SAA) test (Nicolas and Prinssen, 2006), 2) to assess regional brain activity using fMRI in F344 and SD rats. Various brain areas that are associated with anxiety- and stress-related behavioral or autonomic functions were examined as an unbiased approach with the assumption that the brain area showing the largest differences between the strains [i.e. the dmPFC, thought to correspond to the anterior cingulate cortex (ACC) in humans (Seamans et al., 2008; Uylings et al., 2003)] would play a key role in anxiety, 3) to confirm the hyperactivity of the dmPFC in F344 rats with immunolabeling of c-Fos protein, a marker for transcriptional action, and 4) to examine the effects of a neurotoxic lesion of the dmPFC in F344 rats in both the SAA test and fMRI.

2. Experimental procedures

2.1. Animals

All animal procedures were conducted in strict adherence to the Swiss federal regulations on animal protection and to the rules of the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), and with the explicit approval of the local veterinary authority. Male F344 and SD rats (RCC, Switzerland; Iffa Credo, France, respectively), weighing 250 g at the beginning of the experiment, were housed individually. According to an established protocol for the SAA test (Nicolas and Prinssen, 2006), stimulus SD rats (Iffa Credo, France) weighing 450−500 g were kept isolated for several weeks to months and were used in several different experiments (total of eight experiments). All animals were housed under standard maintenance conditions (12:12 h light/dark cycle with lights on at 6 a.m., 21−23 °C, 55−65% relative humidity) and provided with food and water ad libitum.

2.2. Social approach avoidance test

The experimental unit was a box divided in two sections, the non-social (20×40×30 cm) and the social (39×40×30 cm) compartments, connected by a sliding door. The social compartment contained a sub-chamber (14.5×40×30 cm) delimited by a perforated transparent wall confining the stimulus rat. For analysis the two compartments were virtually divided in zones (hidden and protected for non-social; distal and proximal for social). The test started by introducing a test rat into the non-social compartment for a 3 minute habituation period (the stimulus rat was introduced in the sub-chamber just before). At the end of the habituation period, the sliding door was opened allowing the test rat to freely move between the two compartments for 10 min. A tracking system (Ethovision video tracking, Noldus, Netherlands) measured the time spent in different compartment/zones and the distance traveled during habituation as a measure of locomotor activity. For more details, see Nicolas and Prinssen (2006). Group sizes were between 8 and 12 (see figure legends for details).

2.3. fMRI studies

On the day of MRI investigation of regional brain perfusion, animals were anesthetized with an induction level of 4% and a maintenance level of 2−2.5% isoflurane (Abbott, Switzerland) in a mixture of oxygen (0.2 l/min) and air (1.0 l/min) administered via a face mask. Before starting the fMRI measurement, the maintenance isoflurane level was adjusted to standardize the breathing rate to 60 breaths per minute (corresponding to 2−2.5% isoflurane). Rats were positioned in a Plexiglas cradle in the magnet and their heads immobilized in a stereotaxic holder. Body temperature was maintained at 37 °C using a feedback-regulated electric heating blanket. Breathing rate and concentrations of inhaled and exhaled oxygen and CO_2 were continuously monitored on a PowerLab data acquisition system (ADInstruments, Germany). All MRI studies were conducted on a Bruker Biospec 4.7T/40 cm instrument (Bruker Biospin, Germany), equipped with a 12 cm actively shielded gradient set. A 7 cm diameter birdcage coil was used for radio-frequency excitation, and an actively decoupled surface coil was positioned on the head of the animal for signal reception. For all images, the field-of-view was 4 cm and the slice thickness 1 mm.

A set of scout images (T_2-weighted: repetition time (TR)=2.7 s, echo spacing (TE)=10.3 ms, RARE-factor 8, 128×64 matrix, 4 averages) in axial orientation was acquired in each animal, in order to locate the most rostral extension of the corpus callosum, which served as landmark for the subsequent study. Eight coronal image planes which cover all areas of interest at +2.3, +1.0, −0.3, −1.6, −2.9, −5.3, −7.8 and −10.0 mm compared to bregma were selected and a set of anatomical images was obtained from these locations (T_2-weighted: TR/TE=1.8 s/18.0 ms, RARE-factor 8, 256×256 matrix, 4 averages). T_1-maps required to quantify perfusion were also collected using an inversion-recovery-snapshot-FLASH sequence with eight inversion times (TR/TE=7.5 s/1.7 ms, 128×64 matrix, 8 averages) (Haase et al., 1986). Perfusion imaging was conducted based on the continuous arterial spin labeling (CASL) method (Alsop and Detre, 1996; Williams et al., 1992) with a single slice RARE readout module (TR/TE=3 s/5.5 ms, RARE-factor=32, 128×64 matrix, 2 averages, 2.5 s labeling pulse). Each perfusion image plane took