Linking genes to brain function: expression of serotonin 5-HT1A receptors in specific neuronal populations results in divergent phMRI responses to the selective agonist 8-OH-DPAT

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Introduction

Serotonin (5-HT) receptors play a key role in the pathophysiology of anxiety and major depressive disorder [1]. Transgenic mouse lines selectively expressing 5-HT_{1A} receptor in specific brain regions have been recently generated to help investigate the involvement of discrete forebrain circuits in the neuropathological mechanisms underlying these disabling disorders [2,3]. The application of non-invasive imaging techniques such as fMRI to these transgenic lines can provide valuable information as regards the functional contributions of 5-HT_{1A} in discrete components of the serotonergic system. Here we have applied pharmacological MRI (phMRI) to characterize the response to acute pharmacological challenge with the selective 5-HT_{1A} agonist 8-OH-DPAT [4] in serotonin 5-HT_{1A} knockout mice (KO), and in transgenic lines selectively expressing 5-HT_{1A} in the central amygdala (CeA-TG), a key brain region involved in the modulation of affective state.

Methods

All experiments were carried out in accordance with Italian regulations governing animal welfare and protection. Protocols were also reviewed and consented to by a local animal care committee, in accordance with the guidelines of the Principles of Laboratory Animal Care (NIH publication 86-23, revised 1985). **Animals:** The generation of transgenic lines selectively expressing 5-HT_{1A} in the central amygdala (CeA), or completely devoid of the receptor (KO) has been previously described [2,3]. Aged-matched control littermates were used as reference wild-type background. Imaging studies were performed on male subject (22-26 g). **Animal preparation:** Mice were anaesthetised with isoflurane, tracheotomised, and a PE cannula was inserted in the left femoral artery to allow administration of compounds, continuous blood pressure monitoring, and measurement of arterial blood gases. Image acquisition was performed under 1.2% isoflurane anaesthesia, neuromuscular blockade and artificial ventilation. **Experimental groups:** n=10 wild type, n=7 KO, and n=7 CeA mice were challenged with vehicle (saline i.a., 5 µl/g) and 30 minutes later with 8-OH-DPAT (0.5 mg/kg i.a.). Physiological parameters (blood pressure, heart rate, blood gases) were recorded throughout the experiments. **PhMRI acquisition protocol:** PhMRI time series data were acquired on a Bruker Biospec 4.7T system using a T2-weighted RARE sequence (matrix 128x128; FOV 40mm; slice thickness 0.75mm; 16 contiguous coronal slices; RARE factor 32; TEeff=110ms; TR=5121ms; δt=40s (2 averages) in the presence of a blood-pool contrast agent (Endorem, Guerbet, France, 3.75 µl/g) in order to sensitise signal changes to alterations in relative cerebral blood volume (rCBV), as described in detail elsewhere [5]. **Data analysis:** RCBV time series data were spatially normalised to a reference study template, and individual subject response amplitude maps were calculated within the framework of the general linear model using FEAT (FMRI Expert Analysis Tool) Version 5.63, part of FSL (www.fmrib.ox.ac.uk/fsl) and using a model function identified by Wavelet Cluster Analysis (WCA)[6]. 8-OH-DPAT or vehicle administration did not produce significant changes in arterial blood pressure.

Results and discussion

Figure 1 summarises the main findings of this study. Acute administration of 8-OH-DPAT in *wild type* mice produced widespread *deactivation* of the cortex and hippocampus, consistent with the hypothesis of a predominant inhibitory function of 5-HT_{1A} receptors in the normal brain [7]. Mice devoid of 5-HT_{1A} receptors (KO) did not present significant phMRI response to 8-OH-DPAT, a finding that corroborates the selectivity of the pharmacological challenge used. Interestingly, 8-OH-DPAT produced robust and sustained *activation* of the cortex and hippocampus of CeA mice. This result highlights a divergent functional role of distinct receptor populations belonging to the same neurotransmitter system. As presynaptic 5-HT_{1A} autoreceptors are only expressed in medial and dorsal raphe nuclei [8], the positive response observed in CeA-TG is in all probability the result of post-synaptic 5-HT_{1A} receptor activation. Projections from the CeA to cholinergic neurons of the nucleus basalis of Meynert [9] may mediate the marked and widespread cortical activation observed in the CeA line. The presence of cortical activation is consistent with results of behavioural studies showing that intra-amygdala injection of 8-OH-DPAT can produce anxiogenic effects in freely-moving rodents [10]. The results of this study demonstrate the potential of phMRI as a tool to phenotype genetically engineered animals and to characterize *in vivo* the functional role of selectively expressed receptors in specific neuronal circuits.

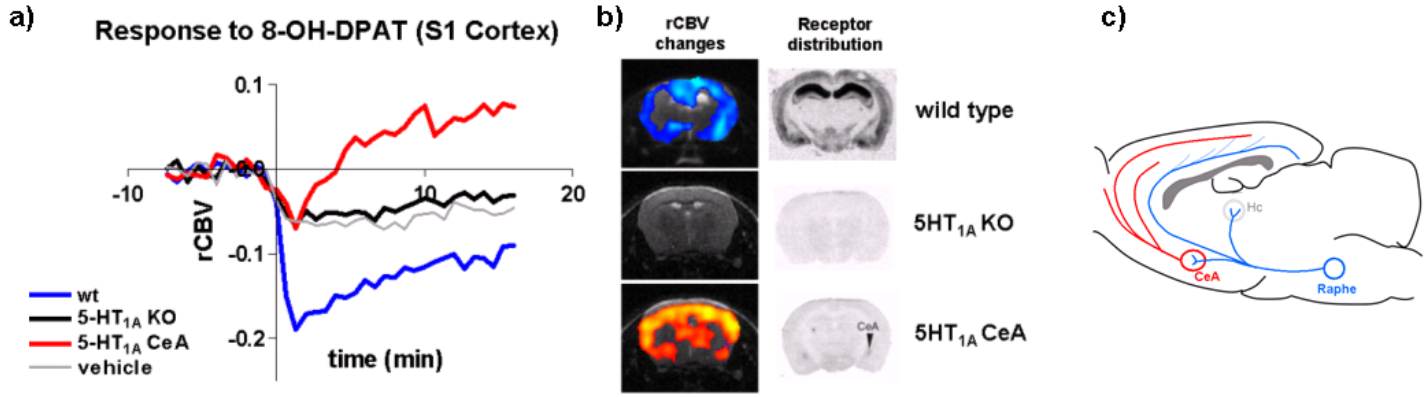

Figure 1 a) *Temporal profile of the rCBV response to the selective 5-HT_{1A} agonist 8-OH-DPAT (0.5 mg/kg i.v.) in the somatosensory cortex. 8-OH-DPAT or vehicle were injected at time 0* **b)** *Anatomical distribution of the rCBV response to 8-OH-DPAT and 5HT_{1A} receptor distribution [from 2,3] in a representative brain slice. Yellow/Orange indicate increased rCBV versus vehicle baseline; blue indicates reduced rCBV versus vehicle baseline. Z (Gaussianised T/F) statistic images were thresholded using clusters determined by Z>3.1 and a corrected cluster significance threshold of p=0.01.***c)** *schematic representation of the serotonergic projections from the raphe nuclei and from the CeA (through the nucleus basalis [8]) to the cortex [CeA: central amygdala].*

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