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Mapping of CBV changes in 5-HT$_{1A}$ terminal fields by functional MRI in the mouse brain

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Abstract

Visualization of brain activity in humans and animals using functional magnetic resonance imaging (fMRI) is an established method for translational neuropsychopharmacology. It is useful to study the activity of defined brain structures, however it requires further refinement to allow more specific cellular analyses, like for instance, the activity of selected pools of brain cells. Here, we investigated brain activity in serotonergic pathways in the adult mouse brain by using acute pharmacological challenge of 5-hydroxytryptamine (5-HT) 1A receptors. We show that administration of the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT prompts a dose-dependent reduction in local cerebral blood volume (CBV) in brain areas rich in neurons expressing post-synaptic 5-HT$_{1A}$ receptor, including the prefrontal cortex, hippocampus and amygdalar nuclei. Region-specific inhibition of the response by co-injection of 8-OH-DPAT with the selective 5-HT$_{1A}$ receptor antagonist WAY-100635, or in 5-HT$_{1A}$ knock-out mice, suggests that 5-HT$_{1A}$ receptors are the

Abbreviations: 5-HT-R, 5-hydroxy-tryptamine (serotonergic) receptor; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino) tetralin; BLA, basolateral amygdala; BM, basomedial amygdala; CBV, cerebral blood volume; Cg, cingulate cortex; DHC, dorsal hippocampus; DR, dorsal raphe nucleus; FMRI, functional magnetic resonance imaging; LA, lateral amygdala; LS, lateral septum; PAG, periaqueductal gray; PET, positron emission tomography; RF, radiofrequency; ROI, region-of-interest; SNR, signal-to-noise ratio; SE-RARE, Spin-echo rapid acquisition with relaxation enhancement technique; tcpCO_2, transcutaneously assessed partial pressure of carbon dioxide; VHC, ventral hippocampus; WAY-10063, N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-pyridinyl)-cyclohexane carboxamide.

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primary targets of the agonist. Overall, the data demonstrate the feasibility of mapping region-specific serotonergic transmission in the adult mouse brain *in vivo* by non-invasive fMRI. The method opens novel perspectives for investigating 5-HT$_{1A}$ receptor functions in mouse models of human pathologies resulting from a dysfunction of the 5-HT$_{1A}$ receptor or the serotonergic system, including depression and anxiety.

1. Introduction

Alterations in serotonin (5-hydroxytryptamine; 5-HT) neurotransmission have been implicated in the pathophysiology of several psychiatric diseases, including depression and anxiety disorders (Garner et al., 2009; Graeff, 2002). The modulatory monoamine serotonin binds to specific transmembrane receptors that belong to a large family (Hannon and Hoyer, 2008). One of the best-characterized receptors is the 5-HT$_{1A}$ receptor which is coupled to inhibitory G-proteins, and is implicated in the etiology of depression (Blier and Ward, 2003; Neumeister et al., 2004; Albert and Lemonde, 2004). In the mammalian brain, 5-HT$_{1A}$ receptors are localized both, presynaptically as somato-dendritic autoreceptors in the raphe nucleus, and post-synaptically in prefrontal and frontal cortex, septal nuclei, periaqueductual gray and limbic areas such as hippocampus and amygdala (Hoyer et al., 1986; Laporte et al., 1994; Bockaert et al., 2006). This pre- versus post-synaptic distinction is functionally important because pre- and post-synaptic receptor populations mediate different responses. Activation of 5-HT$_{1A}$ autoreceptors by administration of a 5-HT$_{1A}$ receptor agonist such as 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT, aminotetralin) (Arvidsson et al., 1981), decreases the firing rate of serotonergic neurons in hippocampus and cortex, and reduces the level of 5-HT release in these areas (Sprouse and Aghajanian, 1988; Hjorth and Sharp, 1991). In contrast, post-synaptic receptors play a modulating role by regulating the activity of non-serotonergic neurons in 5-HT terminal fields. Under physiological conditions, neurons in the raphe nucleus remain under the functional control of projection neurons located in the medial prefrontal cortex, in part through post-synaptic 5-HT$_{1A}$ receptors (Celada et al., 2002).

In vivo functional measurement of 5-HT$_{1A}$ receptor activity is an attractive method to evaluate the role of 5-HT$_{1A}$ receptors in the pathology of psychiatric disorders. For instance, the density of 5-HT$_{1A}$ receptors in brain regions implicated in emotional regulation and response to stress was imaged in patients with depression or anxiety disorders using positron-emission-tomography (PET), and was found to be decreased (Sullivan et al., 2005; Lanzenberger et al., 2007; Kumar and Mann, 2007; Hirvonen et al., 2008; Savitz et al., 2009). However, analogous PET studies in mouse models of psychiatric disorders are less established. So far, only the fluorinated tracers [^{18}F]FCWAY and [^{18}F]FPWAY, analogues of the 5-HT$_{1A}$ receptor antagonist WAY100635 were evaluated *ex vivo* in mice (Jagoda et al., 2006). In general, meaningful small animal PET experiments are still limited by the availability of suitable PET tracers and a spatial resolution of >1 mm^3. Furthermore, PET measurements only provide information on a given receptor expression while fMRI measures the functional consequences of the receptor stimulation which can be achieved at much higher resolution compared to PET.

Pharmacological fMRI is a powerful method that allows monitoring of the acute effects of specific receptor ligands (agonists or antagonists) on brain activity. A large majority of pharmacological fMRI studies in animals have been carried out in rats and analyzed in regards to both the magnitude and spatial extent of fMRI responses for dopaminergic (Chen et al., 1997), GABAergic (Reese et al., 2000), glutamatergic (Houston et al., 2001; Jones et al., 2005), or serotonergic (Houston et al., 2001) neurotransmission. The concept was also successfully applied to animal models of neurodegenerative and psychiatric dysfunctions, and has become a common method for the preclinical characterization of CNS drugs (Rudin et al., 2003; Borsook et al., 2006; van der Linden et al., 2007; Martin and Sibson, 2008). For the 5-HT system, most fMRI studies (Houston et al., 2001; Stark et al., 2006; Hackler et al., 2007; Stark et al., 2008) have focused on the 5-HT$_{2C}$ receptor using the 5-HT$_{1B/2C}$ receptor agonist *meta*-chlorophenylpiperazine (*m*-CPP), essentially because this receptor is a major target for novel potential anxiolytic drugs (Wood, 2003). Only one study examined the 5-HT$_{1A}$ receptor so far. In this study, the acute administration of the agonist 8-OH-DPAT in rat was shown to decrease CBV in several brain areas, in particular the hippocampus and septum (Scanley et al., 2001).

Pharmacological fMRI in the mouse has not yet often been used despite the fact that genetically engineered mice are powerful models of brain disorders. The feasibility of fMRI in the mouse has nonetheless already been demonstrated in transgenic models of Alzheimer's disease (Mueggler et al., 2002, 2003) and schizophrenia (Kuriwaki et al., 2004). However, it is still limited by the requirement of a higher spatial resolution due to smaller brain size and for an increased sensitivity of signal detection. The use of cryogenic radio-frequency (RF) detector devices for mouse brain studies has emerged as a cost effective solution for increasing such sensitivity. It allows a 2–2.5 fold gain when compared to RF receiver operating at room temperature (Ratering et al., 2008; Baltes et al., 2009). Additionally, compared to fMRI studies in the rat, the experimental conditions needed to provide stable physiological parameters for mouse studies are demanding (Mueggler, 2006).

In this study, our aim was to show the feasibility of monitoring CBV response, as a representation of 5-HT$_{1A}$ receptor-mediated neuronal activity, after challenge with the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT in the adult mouse brain *in vivo*. We aimed at detecting distinct decreases in CBV correlating with the region-specific expression of the 5-HT$_{1A}$ receptor. The specificity of the pharmacological manipulation was validated using the 5-HT$_{1A}$ receptor antagonist N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-pyridinyl)-cyclohexane carboxamide (WAY-10063) (Laporte et al., 1994), and a knock-out mouse model deficient for 5-HT$_{1A}$ receptor.