Mapping the circuit of fear with pharmacogenetic silencing and fMRI

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Introduction

Recent advancements in mouse genetics have led to the development of methods to induce transient and cell-type-specific silencing of neuronal activity *in vivo* [1]. In combination with behavioural observations, this novel approach provides a powerful means to assess the functional contributions of these neurons to specific behaviours [2]. Here we demonstrate for the first time the combination of functional MRI (fMRI) and tissue-specific pharmacogenetic silencing to spatially resolve behaviour-specific circuits controlled by discrete neuronal populations focally expressed in the mouse brain. Specifically, we examined the effect of inhibition of neural activity in a subset of neurons of the central nucleus of the amygdala (CeA), a key structure involved in the control of emotional and fear responses [3]. Reversible suppression of neural activity in a subset ("Type-I") of CeA neurons was achieved by inducing tissue-specific re-expression of the serotonin 1A receptor (Htr1a) in mice devoid of the endogenous receptor ([2] the resulting mice called *Htr1a*^{CeA}). Systemic administration of the selective Htr1a agonist 8-OH-DPAT produces rapid inhibition of the neurons expressing the receptor [4]. By combining fMRI and this pharmacogenetic inhibition strategy we mapped the downstream functional effects of CeA silencing, and correlated them with competing behavioural responses to aversive stimuli in a fear conditioning paradigm.

Methods

All experiments were carried out in accordance with Italian regulations governing animal welfare and a local animal care committee **Animal preparation:** Male (22-26 g) mice were anaesthetised with isoflurane, tracheotomised and artificially ventilated under neuromuscular blockade. Left femoral artery was cannulated to allow administration of compounds, continuous blood pressure and arterial blood gases monitoring. Image acquisition was performed under 1.2% isoflurane anaesthesia. **Experimental groups:** *Htr1a*$^{+/+}$ (wild-type, N=14), *Htr1a*^{KO} (N=8) or *Htr1a*^{CeA} (N=9) were challenged with vehicle (saline, 5 µl/g) and 8-OH-DPAT (0.5 mg/kg i.a.) 30 min later. Two additional groups of *Htr1a*^{CeA} (N=5 each) or *Htr1a*$^{+/+}$ (N=8) received an injection of cholinergic antagonist atropine-sulphate or methyl-nitrite (0.5 mg/kg i.p.) 15 min prior to 8-OH-DPAT administration. **fMRI acquisition :** Images were acquired on a Bruker Biospec 4.7T using a RARE sequence (128x128x16 slices, thickness 0.75mm; FOV 40mm; RARE factor 32; TEeff=110ms; TR=5121ms; δt=40s). Blood-pool contrast agent was administered (3.75 ul/g) to measure microvascular relative Cerebral Blood Volume (rCBV) changes [5]. **Data analysis:** fMRI time-series were spatially normalised to a reference study template, and individual subject response amplitude maps calculated within the framework of the general linear model using FEAT Version 5.63, and using model functions identified by Wavelet Cluster Analysis (WCA)[6].**Behavioural experiments:** Fear conditioning testing was carried out as previously described [2]. All behaviours were recorded as total duration of the activity.

Results and Discussion

Administration of 8-OH-DPAT led to significant rCBV decreases in all structures where Htr1a is expressed (Z>2.3), while no significant rCBV changes were observed in Htr1a^{KO} mice (Z>1.6). These data indicate that neural inhibition associated with activation of Htr1a can be mapped *in vivo* using fMRI. Interestingly, suppression of type-I CeA neurons led to widespread activation of several forebrain areas (Z>3.5). In order to map downstream nuclei that mediate cortical activation in *Htr1a*^{CeA} mice, we applied correlation analysis [7] to the regional fMRI responses. A significant pattern of correlated activity was identified linking CeA and cortical regions most strongly activated by 8-OH-DPAT with cholinergic nuclei in the ventral forebrain. The involvement of cholinergic neurotransmission was demonstrated in a separate experiment where the acetylcholine receptor antagonist atropine-sulphate (but not a poorly brain-penetrant methyl-nitrite salt) potently suppressed 8-OH-DPAT induced activity. Importantly, atropine sulphate did not attenuate the rCBV response to 8-OH-DPAT in wild type mice (Z>1.6). These results suggest that suppression of neural activity in type-I CeA neurons leads to arousal of cortical circuits through disinhibition of cholinergic nuclei. Finally, we examined the behavioural correlates of the observed cortical arousal by measuring conditioned behavioural responses elicited by the presentation of conditioned aversive stimulus as described in [2]. *Htr1a*^{CeA} mice treated with 8-OH-DPAT exhibited significant reduction of freezing behaviour that was inversely correlated with a dramatic increase in active exploratory and risk-assessment behaviours. These data suggest that Type-I neuron in the CeA act a switch between active and passive fear-coping strategies.

Conclusion: Our data provide a compelling demonstration of the combined use of fMRI and cell-type specific neural inhibition as a new powerful paradigm to identify and resolve behaviour-specific neural circuits *in vivo*.

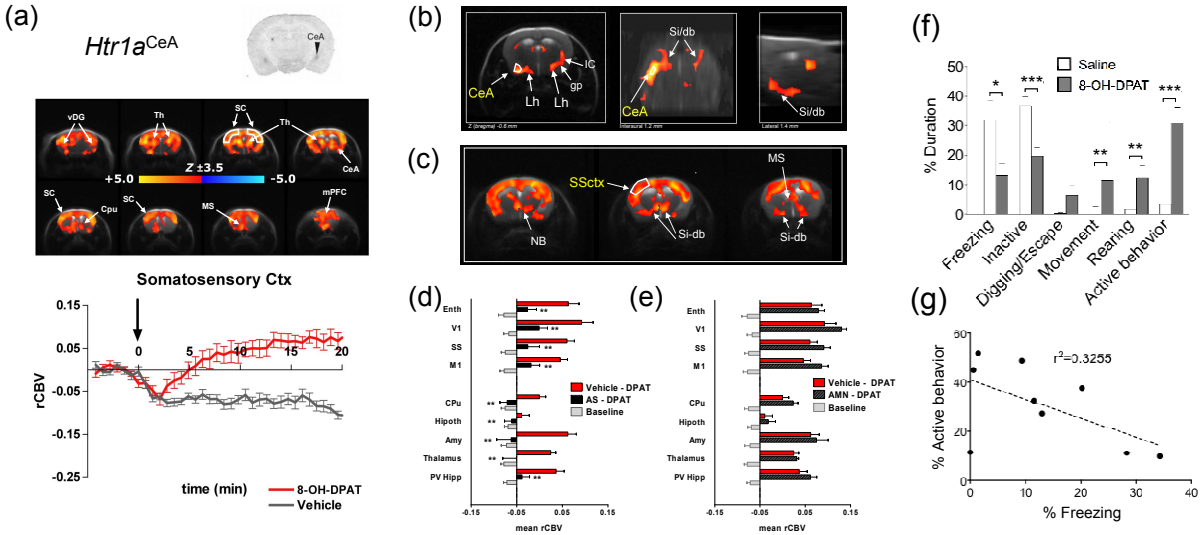

Figure 1 (a) Cortical arousal following suppression of type-I CeA cells. Anatomical distribution (and representative timecourse, bottom) of the rCBV changes in *Htr1a*^{CeA} mice treated with the agonist 8-OH-DPAT. Orange indicates *increased* rCBV versus vehicle (Z>3.5) **(b,c) Ventral forebrain cholinergic neurons are a downstream target of the CeA** Maps of 8-OH-DPAT-induced rCBV response significantly correlated with CeA (b) or somatosensory cortex (c) in *Htr1a*^{CeA} mice (Z>1 .6; LH: lateral hypothalamus Gp: globus pallidus; IC: internal capsule; Si: substantia innominata; db: diagonal band of Broca; MS: medial septum NB: nucleus basalis of Meynart) **(d,e) Cortical arousal depends on central cholinergic neurotransmission.** Pretreatment with atropine sulphate (AS, **d**) but not the non-brain penetrant salt AMN, **(e)** suppressed the mean rCBV response to 8-OH-DPAT (**p<0.01 versus vehicle). **(f, g) Decreased passive and increased active behavioral responses to conditioned aversive stimulus.** Pharmacogenetic silencing with 8-OH-DPAT increased active behavioural responses to a fear-conditioning paradigm at the expense of passive behaviours (i.e. freezing, ***p<0.01 vs. saline).

References [1] Luo et al., 2008 [2] Tsetsenis et al., 2008 [3] LeDoux et al., 1998 [4] Luscher et al., 1997 [5] Mandeville et al., 1998 [6] Schwarz et al., 2007a [7] Schwarz et al., 2007b