Intransal administration of neuroactive peptides elicits robust fMRI responses in the mouse brain

Alberto Galbusera[1], Angelo Bifone[1], and Alessandro Gozzi[1]
[1]*MRI Laboratory, Istituto Italiano di Tecnologia, Centre for Neuroscience and Cognitive Sciences, Rovereto, Trento, Italy*

Introduction

Treatment of central nervous systems is complicated by the presence of the blood brain barrier (BBB), an endothelial layer of cells that impedes influx of large molecular weight molecules from blood to brain. Recent studies have highlighted the possibility of exploiting intranasal administration as non invasive method for direct CNS delivery of neuro-active peptides and large biological therapeutics. The approach is the object of extensive pre-clinical and clinical investigation, with encouraging preliminary results (*1*). However, the dynamics and neurofunctional substrates recruited upon nasal administration of neuroactive peptides remain unexplored.

Pharmacological fMRI (i.e. the use of fMRI to spatially-resolve drug-elicited functional signals) is being increasingly used to map the circuits engaged by pharmacological agents and obtain early translational evidence of mechanistic engagement (*2*). However, no attempt to use the technique to map the circuital substrates *directly* modulated by intranasally-administered biologicals has been described. Here we used high-resolution fMRI in the mouse to map the functional response elicited by nasal administration of behaviourally-active doses (*3*) of two well-characterised neuropeptides (oxytocin and vasopressin) that are being proposed as adjunctive therapy for neuropsychiatric diseases characterised by social impairments (e.g. autism spectrum disorders and schizophrenia)(*4, 5*).

Methods

All animal procedures were in compliance with institutional animal care and use committee guidelines: European Guidelines for Animal Care and Use of Experimental Animals 2010/63/EU. **Animal preparation**: Male C57BL6/J mice were prepared as previously described (*6*). Briefly, they were anaesthetised with isoflurane, intubated and artificially ventilated under neuromuscular blockade. Left femoral artery was cannulated to allow continuous blood pressure monitoring and arterial blood gas sampling. Image acquisition was performed under 0,9% halothane anaesthesia. **MRI acquisition:** MRI data were acquired using a Bruker 7 T scanner. fMRI times series were acquired using a FLASH MRI sequence (TR = 288 ms, TE = 3.1 ms, α=30°; 180 x 180 x 600 µm resolution, dt = 60 s, Nr = 60). Images were sensitized to reflect alterations in rCBV (*7*) upon injection of 5 µl/g of blood-pool contrast agent (Molday Ion, Biopal). **Intranasal peptide administration:** Two PTFE cannulas were inserted in nasal cavity as previously described (*8*). Mice were randomised and challenged with vehicle (water, 4 ul/mouse, N=7) or Oxytocin (OXT, 1.33 µg/mouse, N=8) or Vasopressin (1.33 µg/mouse N=7). **Data analysis:** fMRI timeseries 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 capturing the sustained response produced by the peptides.

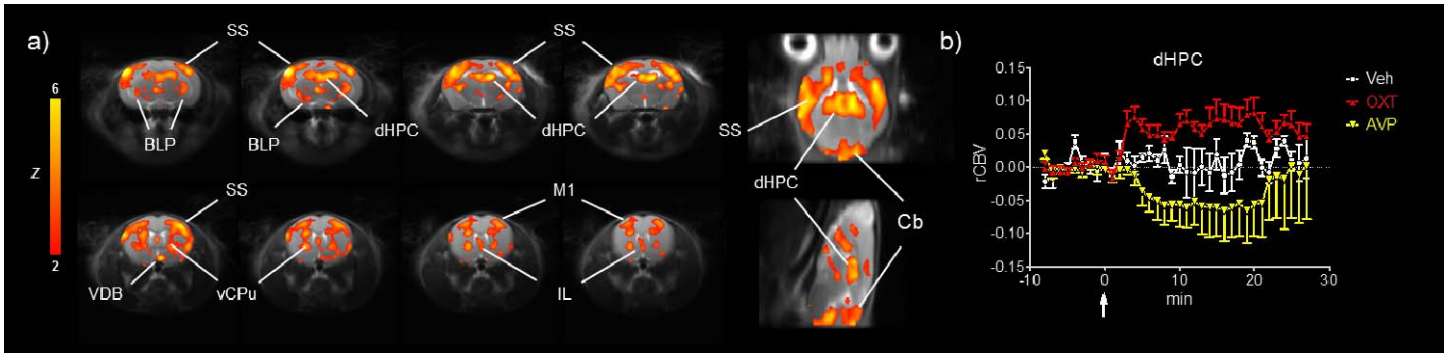

Figure 1 ***a)*** *Anatomical distribution of the rCBV response to intranasal administration of Oxytocin (1.33 ug/mouse) in representative brain slices. Yellow/Orange indicate increased rCBV versus vehicle baseline (Z>2, cluster correction, p=0.01) [abbreviations: BLP, basolateral nucleus of the amygdala; SS, somatosensory cortex; dHPC, dorsal hippocampus; VDB, nucleus of the vertical limb of the diagonal band; vCPu, ventral portion of caudate putamen; M1, primary motor cortex; IL, infralimbic cortex; Cb, cerebellum]* **b)** *Temporal profile of the rCBV response to Oxytocin (OXT; 1.33 ug/mouse) and Vasopressin (AVP; 1,334 ug/mouse) in the dorsal hippocampus. The peptides were injected at time 0.*

Results & Discussion

Figure 1 summarises the main findings of this study. Acute administration of a behaviorally-active dose of Oxytocin (*3*) produced rapid and robust activation of a composite network of cortico-limbic regions comprising the dorsal hippocampus, motor, infralimbic and parietal cortex, ventral striatum, diagonal band and posterior amygdaloidal nuclei, plus many periventricular regions. A robust response was also observed in cerebellar areas. Several of these regions are characterized by high Oxytocin receptor density (*9, 10*). The hippocampal activation was especially robust and prominent, and is consistent with a key contribution of this hormone as a facilitator of information processing in the brain (11). Oxytocin administration produced a slight and transient increase in arterial blood pressure.

Nasal administration of a similar amount of vasopressin produced widespread deactivation of cortical and subcortical regions, including multiple neocortical regions, hippocampus, thalamus and striatum. The opposing effect of oxytocin and vasopressin is in keeping with the divergent neurobehavioral profile of the two peptides (*12*). Vasopressin produced a robust increase in blood pressure, an established neurophysiological signature of this peptide (*13*). A central contamination of the peripheral response in unlikely, owing to the presence of intact autoregulation under the experimental conditions of our study (*14*). Moreover, hypertensive responses exceeding the autoregulation window would result in *increased* rCBV (*14, 15*). The rapid onset of the effects mapped are consistent with the current hypothesis of a combined intra-axonal (low action) and perineuronal transport of the peptide via cerebrospinal fluid (*10*). Our study represents the first demonstration of the possibility to non invasively map the substrates recruited by intranasally-administered peptides and pave the way to the use of this approach to the characterization of CNS effects of centrally-active biologicals that do not cross the blood-brain barrier

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