Drug-anaesthetic interaction in pharmacological MRI: the case of the psychotogenic agent phencyclidine

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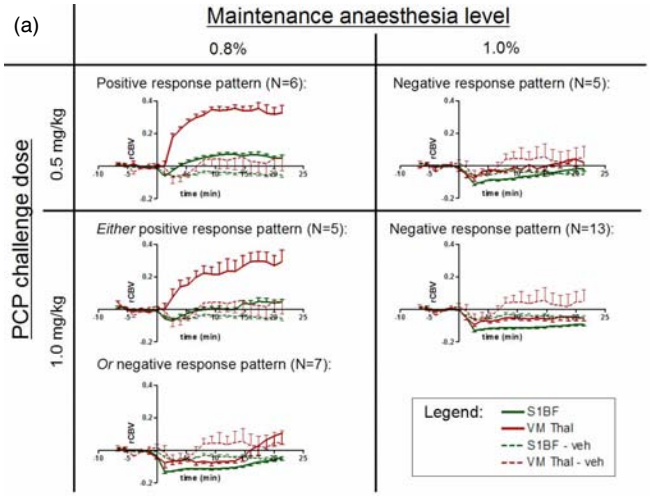
Introduction Acute administration of sub-anaesthetic doses of phencyclidine (PCP) induces psychotic behaviour in humans and laboratory animals and is a widely-used neuropharmacological model of schizophrenia [1]. However, the neuronal substrate underlying these effects is still the subject of active investigation. Here, we applied pharmacological MRI (phMRI) methods to elucidate the brain circuitry underlying the psychotomimetic action of PCP using an rCBV-sensitive protocol in the halothane-anaesthetised rat. Interestingly, we found that both the anatomical distribution and the direction of the response dramatically depended on both anaesthetic level and challenge dose. We applied a factorial study design to investigate this non-trivial interaction between PCP and anaesthetic agent and guide the selection of an appropriate combination of PCP dose and anaesthetic regimen.

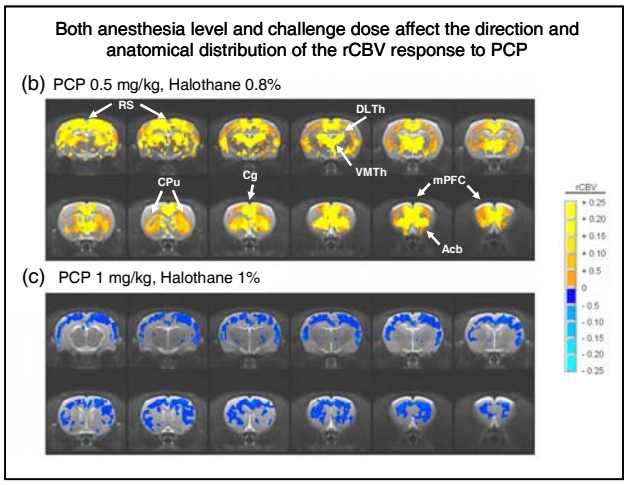
Methods All experiments were carried out in accordance with Italian regulations governing animal welfare and protection. Protocols were also reviewed and consented to by a local animal care committee, in accordance with the guidelines of the Principles of Laboratory Animal Care (NIH publication 86-23, revised 1985).

Study design: N=40 male SD rats were surgically prepared as detailed previously [2] and imaged under halothane anaesthesia, neuromuscular blockade and artificial ventilation. Animals were assigned to one of four arms, corresponding to the different combinations of two PCP challenge doses (0.5mg/kg or 1.0mg/kg i.v., nr. of subjects per group reported in Fig 1(a)), or its vehicle (saline, n=4), and two maintenance anaesthesia levels (0.8% or 1.0% halothane). Physiological parameters were monitored throughout the experiments to assess the level of anaesthesia. Moreover, a pilot study was performed without neuromuscular blockade to verify that anaesthesia levels were adequate for maintenance under all experimental conditions used in this study.

PhMRI acquisition protocol: PhMRI time series data were acquired on a Bruker Biospec 4.7T system using a T_2-weighted RARE sequence (RARE factor 32, matrix 128x128, FOV 40mm, slice thickness 1mm, 16 contiguous coronal slices, TR_{eff} = 2700ms, TE_{eff} = 100ms) in the presence of a blood-pool contrast agent (Endorem, Guerbet) in order to sensitise signal changes to alterations in relative cerebral blood volume (rCBV), as described in detail elsewhere [3;4].

Data analysis: RCBV time series data were spatially normalised to a stereotactic rat brain template[5]. Individual subject response amplitude maps were calculated within the framework of the general linear model [6]. Blood pressure response to PCP was modest, transient, not correlated with the time-profile of the rCBV changes, and well within the range of cerebral blood flow autoregulation under halothane anaesthesia [7].





Results The different combinations of PCP dose and anaesthetic level resulted in striking differences in the anatomical location and direction of the rCBV response, with two qualitatively distinct profiles (Fig. 1). In the {0.8% HT, 0.5mg/kg PCP} arm, focal positive rCBV changes were observed, in limbo-thalamic and prefrontal/cingulate cortical regions (Fig. 1(a,b)). At the higher maintenance anaesthetic level, in both {1.0% HT, 0.5mg/kg PCP} and {1.0% HT, 1.0mg/kg PCP} arms, a sustained negative rCBV response localised to cortical regions was observed (Fig. 1(a,c). In the {0.8% HT, 1.0mg/kg PCP} arm the response was strongly subject dependent, with 5/12 animals showing a positive response similar to that of Fig. 1(b) (albeit slightly weaker), and the remaining 7/12 a negative response similar to that of Fig. 1(c).

Discussion and conclusion This study demonstrates that interaction between PCP challenge dose and maintenance anaesthetic level can drastically affect the temporal and spatial response patterns. Two distinct activation patterns, characterised by a different direction and anatomical distribution of the rCBV response were observed. Several lines of evidence suggest that an activation of cortical and limbo-thalamic structures like the one described in Fig. 1(b) may underlie the psychotic-like effects of PCP in non-anaesthetised animals. [14C]-2-deoxyglucose uptake maps obtained in freely moving rats challenged with phencyclidine show a striking degree of similarity with the distribution of the *positive* rCBV response pattern observed in this study.[8-12]. Activation of these structures has also been demonstrated with ex-vivo functional measurements like c-*fos* and [14C]iodoantipyrine CBF measurements[13;14]. Hence, the pattern of Fig.1(b) is a plausible representation of the neural substrate underlying the psychotic effects of PCP. At higher PCP and/or anaesthetic doses, a widespread cortical decrease in rCBV was observed, inconsistent with the existing evidence. As PCP itself acts as a general anaesthetic at sufficiently high doses [15], it is conceivable that PCP and halothane may synergise resulting in a reduction of cortical activity. However, it is surprising that this effect occurs abruptly and that even with small changes in either challenge dose or anaesthetic concentration can result in one or another of two entirely different spatiotemporal patterns of activation. These results further highlight the potentially confounding effects of the anaesthetic agents used in many in phMRI studies and the importance of pilot studies to determine the appropriate experimental conditions, since the interactions of the drug with the anaesthetic agent is not always predictable.

***Figure 1**: (a) Summary of study arms and observed responses. Group time courses (mean +/- SEM) are shown for the somatosensory cortex (S1BF region) and the ventromedial thalamus. Veh: vehicle (b,c) Group activation maps (p_u<0.001) indicating the two characteristic activation signatures observed, depending on the challenge dose and anaesthetic level. Yellow indicates* increased, *blue indicates* reduced *rCBV versus vehicle baseline; RS: restrosplenial cortex(ctx); Cg: cingulate ctx, DL/VM Th: dorsolateral /ventromedial thalamus, Cpu:caudate putamen, mPFC:Medial prefrontal cortex, Acb: nucleus accumbens.*

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