Focal and Drug-Specific Changes in Cerebral Blood Flow in Response to Dose-Controlled Infusion of Alcohol and Morphine in Healthy Young Men

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Introduction: One of the primary questions in central nervous system drug development is to identify brain regions with preferential affinity for drug action. Traditionally, information about site-specificity of drug action is drawn from animal (mostly rodent) models. Translational studies expand these findings to human drug research with costly clinical trials based on a-priori assumptions about the site of action and behavioral outcomes of drug actions. Functional neuroimaging methods such as PET and fMRI have been recently used in CNS drug research. However, limitations to repeat PET in the same subject and task-dependency of most of fMRI studies limit the scope of research to hypothesis-based experiments. These limitations potentially obscure important information outside of the *a priori* hypotheses. If techniques such as continuous arterial spin labeling (CASL) are sensitive enough to reveal site-specific changes in brain perfusion after pharmacological manipulations, then they might offer a repeatable (like fMRI) and quantifiable (like PET) measure of local neural response to different drugs. Previously, we illustrated drug-specific changes in the connectivity of cerebellar, sensory motor and paralimbic brain areas, as observed from examining the topographically correlated changes in resting-state BOLD fluctuations (*abstract submitted to this conference*). In this parallel study, we have used pseudo CASL (p-CASL) in a similar within-subject placebo-controlled repeated measures study to investigate effects of dose-controlled intravenous infusion of alcohol and morphine on local and global CBF changes.

Methods: Twelve healthy young men (18-40 yrs) participated in a double blind, placebo controlled repeated measure study consisting of three examination sessions. In each procedurally identical session, drug compounds (placebo, alcohol or morphine) were administered intravenously and physiological and psychological assessments were done repeatedly. Drug infusion was controlled based on validated pharmacokinetic models to ascertain controlled levels of plasma drug concentration, and minimize between subject variations (E. Sarton *et al.*, *Anesthesiology* **93**; 2000). Before drug injection and 120 minutes after drug infusion, the cerebral blood flow (CBF) was measured using a p-CASL at 3 Tesla (M. J. Van Osch *et al*, *Magnetic Resonance in Medicine* **62**; 2009**).** Thirty read-outs (single shot EPI, 17 slices of 7 mm with an in-plane resolution of 3x3 mm2, SENSE factor 2.5, TE=13.9 ms at a delay of 1525 ms, slice time 35 ms) were obtained (total scan time of 4m10s). For each subject we obtained 6 p-CASL data sets. For each set, voxelwise CBF was computed using $CBF(x,y,z)=\frac{\sum_{t=1}^{N}(S_{control}(x,y,z,t)-S_{label}(x,y,z,t))}{N}\frac{1}{labefficiency\times M_0\times T_1blood\times\lambda}e^{(\frac{delay+slicetime(z-0.5)}{T_1blood}+\frac{TE}{T_2})}$, where N=30; λ=0.76, lab efficiency=0.85, TE=13.9 ms, T_2=50 ms and T_1blood=1680 ms. Using FSL, the CBF maps were normalized to MNI152 template, the global CBF change was obtained by averaging the CBF intensities inside a standardized brain mask. Each CBF map was normalized with its global mean. The resulting maps were then tested with a GLM to identify treatment by time effects. Significance was set at $p < 0.005$, voxelwise; and at $p<0.05$, cluster-corrected.

Results: Treatment effects are summarized in Table 1.Figure 1 illustrates local changes in CBF. Treatment interactions with global CBF changes were not significant, $F(2,33) = 2.77$, $p > 0.10$. Treatment interaction with heart and respiration rates was significant ($F's(2,33) > 8.6$, $p's < 0.005$). Drug related changes in CBF were localized. Compared to placebo, morphine significantly reduced CBF in several somatosensory and motor areas in the left hemisphere. Comparatively, effects of alcohol (ethanol) versus placebo were limited to the cerebellum. We illustrated significant difference in drug action in the cerebellum and the brainstem (morphine > alcohol), and in the hippocampus, putamen as well as precentral, middle temporal, and opercular regions that are linked to consciousness. Similarly, we had observed significant effect of morphine on resting-state connectivity (measured from BOLD fluctuations) of sensory-motor, hippocampal and paralimbic areas (ACC and precuneus). Similarly, alcohol effect was mostly significant on the resting-state connectivity of the cerebellum to somatosensory network.

Conclusion: These results successfully illustrate that p-CASL provides a sensitive method for detecting local changes in cerebral perfusion of pharmacologically stimulated brain. The continuous labeling is advantageous as it tags the whole brain, thus enabling to detect different cerebellar changes effects of each drug. Future experiments need to investigate how drugs modulate the link between CBF and BOLD-related changes in brain function. Anatomical specificity of local changes in the CBF might help formulating better hypotheses for examining the effects of CNS drugs on behavior or their potential side effects.

Table 1	Placebo		Morphine		Ethanol	
	pre	post	pre	post	pre	post
Global mean CBF	33.75±13.84	28.53±11.26	29.9±12.85	35.42±16.76	32.30±12.55	32.22±12.90
Morphine nmol/L	0	0	0	66.88±5.87	0	0
Ethanol mg/L	0	0	0	0	0	0.588±0.062
Heart Rate (bpm)	63.59±9.52	56.4±9.51	62.84±8.56	51.67±10.15	61.49±7.46	58.42±7.62
Respiration (ppm)	15.98±3.47	16.57±2.39	16.13±2.87	13.20±1.60	15.41±3.11	16.27±3.7

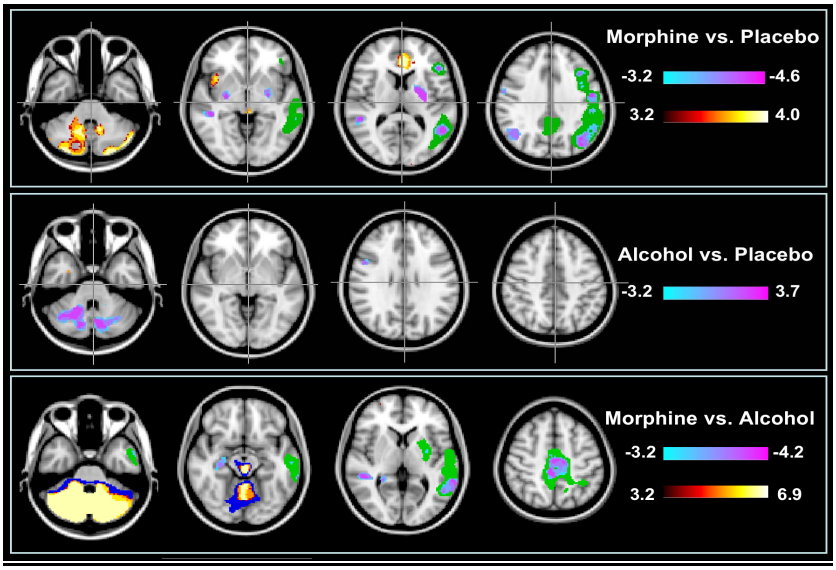

Figure 1: Green (increased CBF) and dark blue (decreased CBF) background areas are cluster-corrected at $p<0.05$. Cool- and hot color areas represent t-values of changes in CBF, at uncorrected $p<0.005$, respectively.