Feasibility of Detecting ABT-594-Induced Brain Activity Using CBV-Based fMRI in Awake and Anesthetized Rats

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Introduction

Anesthetics are often used in fMRI animal studies in order to minimize motion artifacts. Unfortunately, anesthetics, are known to have direct and indirect effects on the nervous and vascular systems. Thus, the use of anesthetics may confound data interpretation following the administration of exogenous pharmacological agents. To avoid anesthetic-related interactions, several groups have explored the feasibility of conducting BOLD and CBF fMRI in awake animals [1-5]. The aims of this study were (1) to examine the feasibility of conducting CBV fMRI studies in awake rats using ABT-594 (a neuronal nicotinic receptor agonist), and (2) to quantitatively compare fMRI baseline fluctuations and sensitivity between awake and anesthetized rats under the same pharmacological intervention.

Materials and Methods

Animal Preparation Male SD rats (250~300g) were studied under awake and anesthetized conditions. To reduce stress, awake rats were acclimated to a dedicated animal restrainer for four sessions (7min/30min/1hr/1hr, on separate days) prior to the actual experiment. Anesthesia was induced using α-chloralose at 65mg/kg (i.p.), then maintained at 20mg/kg/hr (i.v.). For the anesthetized rat studies, arterial blood pressure, heart rate, and blood gas were monitored throughout experiments; rats were excluded from anaysis if physiological conditions were abnormal.

fMRI Protocol and *Data Analysis* Animals were placed and secured in a rat restrainer integrated with a dual RF coil system (Insight Neuroimaging Systems, Worcester MA). Experiments were carried out on a 4.7T Varian scanner using a FLASH sequence with imaging parameters: TR/TE = 450/20ms, flip angle=20°, matrix size=128×64, slice thickness=1.5mm,13 slices and a FOV of 4×4 cm^2. Following 7 minute baseline acquisition, a ultrasmal super paramagnetic iron oxide (USPIO) contrast agent (SH U 555 C, 10 mg Fe/kg, i.v., Schering AG, Berlin, Germany) was administered. Ten minutes after the injection of the contrast agent, ABT-594 (0.3μmol/kg, i.v.) or saline vehicle was infused over a 5-minute period and imaging acquisition continued for another 30 minutes. Activation maps were generated by calculating the cross-correlation coefficient (cc) between time-course rCBV data and a step function using AFNI [6]. To quantitatively assess baseline fluctuations, the coefficient of variance (CoV=standard deviation/mean of the signal intensity, a measure of the degree of variation) was calculated on a pixel-by-pixel basis over the last 7 minute baseline prior to compound infusion. Finally, ABT-594 induced fMRI signal changes and contrast-to-noise ratio (CNR) were calculated over a region of motor cortex.

Results and Discussion

Following ABT-594 administration, significant activations were observed in several brain regions including cingulate, somatosensory, motor, and prefrontal corticies, as well as the cerebellum (for example, see Fig. 1A). No activations were observed in the vehicle group. Figure 1B illustrates the time-course rCBV changes (ΔrCBV) in awake and anesthetized rats over an ROI in the motor cortex. In awake rats, the magnitude and duration of the drug-induced ΔrCBV were significantly higher compared to the anesthetized rats (the area-under-curve for the acute drug response was approximately 7 times greater) and the average ΔrCBV was found to be 2.92 times larger (Table 1).

Figure 2 shows typical CoV maps calculated from awake and anesthetized rats. Motion artifacts were significantly higher under awake conditions. The mean and standard deviation of CoV (CoV_μ and CoV_σ) over the brain parenchyma were listed in Table 1 (n=10, for both groups). The CoV_μ of conscious rats was 1.9 times higher as compared to the anesthetized rats, indicating significantly higher baseline fluctuation in awake rats (p<0.001). While motion was a major factor contributing to the baseline noise, an increased basal neural activity and/or more sensitized neurovascular coupling under awake conditions may also contribute to the increased fluctuations [3]. Despite the increase in baseline fluctuations, the ratio of CNR for awake versus anesthetized was 1.65, which parallels a study using hypercapnic-challenged rats [3]. Further, the variation in CoV from different brain regions (CoV_σ) was also much larger in awake rats than anesthetized rats (p<0.001), which might suggest that the sensitivity will also vary more from region to region under awake condition.

Table 1 The average ΔrCBV and CNR from the ROI shown in Fig.1A and the calculated CoV_μ and CoV_σ from anesthetized and awake rats.

	ΔrCBV [%]	CNR	CoV_μ [%]	CoV_σ [%]
Anesthetized	11.71	4.12	4.19 (±0.7)	2.72 (±1.1)
Awake	47.47	6.79	12.02 (±3.8)	6.71 (±2.5)

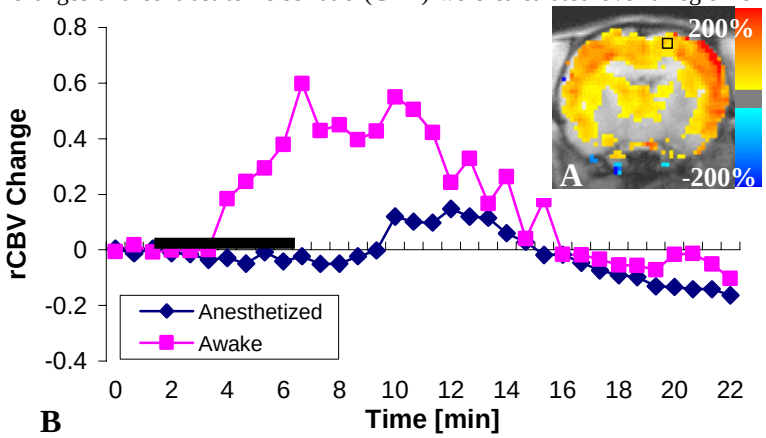

Fig. 1 (A) Typical activation map calculated based on the cross-correlation coefficient (threshold: cc=0.5, p<0.0001). (B) Averaged time-course ΔrCBV data within an ROI in the activated motor cortex region indicated by the box (3×3 matrix) in (A), from awake (n=3) and anesthetized (n=2) rats. The black bar indicates the time of drug infusion.

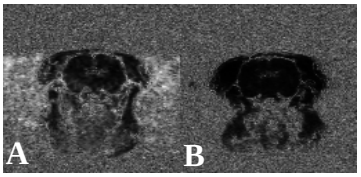

Fig. 2 (left) CoV maps calculated from awake (A) and anesthetized (B) rats. Notably, significant motion artifacts were observed in the awake rat.

Conclusions

We have demonstrated the feasibility to observe ABT-594-induced activations using CBV fMRI on awake and anesthetized rats. Although baseline fluctuations were larger, higher sensitivity can be achieved in awake animals. Thus, fMRI in awake animals provides great potential for studying drug-induced brain activities.

References

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