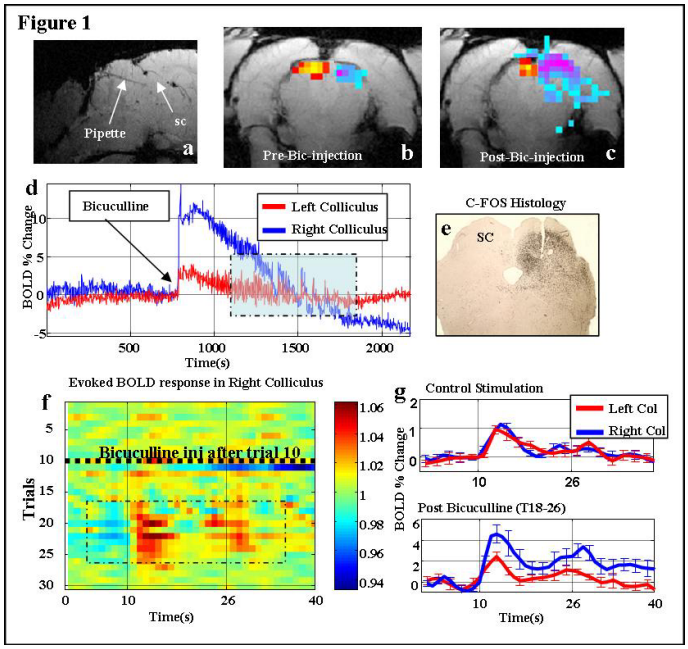
Changes in BOLD signaling induced by local chemical activation of the dorsal midbrain in rats

J. Berwick[1], A. Kennerley[1], C. Martin[1], M. Jones[1], L. Boorman[1], Y. Zheng[1], J. Mayhew[1], and P. Redgrave[1]
[1]Psychology, University of Sheffield, Sheffield, South Yorkshire, United Kingdom

Introduction:

The advent of fMRI has revolutionized brain research in humans. Regionally specific changes in BOLD signaling associated with sensory, motor, cognitive and motivational performance have been recorded at locations throughout the human brain. However, the manner by which components of the circuits identified by BOLD signaling interact to achieve functional output has been rather more difficult to discern. Part of the problem is that in humans, it is not often possible to determine the consequences of direct manipulations of relevant circuitry – hence a pressing need to develop relevant functional models in animals. The purpose of the present study was, therefore, to establish the technology and procedures for measuring the consequences of direct, local chemical stimulation of the brain using fMRI. Our previous work (Dommett et al 2005) has established that the superficial layers of the retinorecipient midbrain superior colliculus in rodents remain visually responsive under anaesthesia. However, local chemical stimulation is required to restore visually evoked activity in the deep layers of this structure. Moreover, following chemical stimulation of the deep collicular layers other areas of the brain targeted by this region also become responsive to visual stimulation. We have, therefore, used this established animal model to develop procedures for measuring the consequences of local chemical stimulation of the brain with fMRI.



Methods:

All aspects of these methods and their development were performed with UK Home Office approval under the Animals (Scientific Procedures) Act 1986. Female Hooded Lister rats (250-400g) were anaesthetised with urethane and body temperature maintained at 37˚C throughout surgical and experimental procedures. The femoral vein and artery were cannulated to allow drug infusion and measurement of mean arterial blood pressure. A small 50µm-diameter glass injection pipette filled with bicuculline methiodide (50ng/500nl saline) was placed into the right superior colliculus (angle of entry 30° from horizontal – Fig. 1a). Bicuculline is an antagonist of the inhibitory neurotransmitter GABA and causes neural excitation by disinhibition. Animals were secured in the magnet by a custom-built Perspex stereotaxic frame. Animals were artificially ventilated and blood pressure monitored throughout. A 20mm surface coil (detailed below) was secured directly above the animal's head. Eye cups fitted with fibre optic light guides were placed over each eye. Whole field visual stimulation was achieved by connecting the fibre optics to individual white light LEDs outside the magnet. The visual stimulation consisted of 16s trains of 50ms light flashes at 10Hz presented to each eye. The experimental schedule consisted of 60 alternating interleaved stimuli (left then right eye) with a 35s inter-stimulus interval. This schedule was repeated, except that after 10 stimuli to each eye, bicuculline (500nl/60s) was injected unilaterally into the right superior colliculus. After all experimental testing animals were killed, perfused with fixative and the brains processed for Fos-like immunoreactivity; c-fos is a marker of neural activation.

MRI Scanning Parameters: An ^{1}H quadrature volume resonator (Bruker 1P-T9561, 300MHz, 1kW max, outer diameter 200mm/ inner diameter 200mm) was placed at the iso-centre of a 7 Tesla magnet (Bruker BioSpec^{AVANCE}, 310mm bore, MRI system B/C 70/30). A 20mm (inner diameter) ^{1}H receiver, surface coil (Bruker 1P-T7399) was connected to a Multlink^{TM} ^{1}H preamplifier (HPPR/2).The volume and surface resonators were tuned and matched to 300MHz using the Multlink^{TM} ^{1}H preamplifier which included a built in tune/match display. Both coils were then connected to an active decoupling coil control unit operated from the console using a DC/pulse-in to ensure accurate transmit/receive times. Functional data were acquired from nine 1mm coronal slices from the whole brain using a single shot MBEST Gradient Echo - Echo Planar Imaging (GE-EPI) sequence during stimulation (raw data matrix = 64*64, data sampling interval = 5µs, FOV = 30mm, slice thickness = 1mm, TR/TE=1000/12ms, flip angle 90^{o}, 10 dummy scans). Read-out direction was left-right for both slice orientations. Standard phase correction (Bruder et al 1992) was used to minimise Nyquist ghosting. The *BOLD* signal was calculated as fractional change normalised by the mean of the 60s data acquisition before the beginning of light stimulation.

Summary of Results:

The 16s trains of light flashes delivered to each of the eyes alternatively, evoked a BOLD response centered on the superficial layers of the contralateral colliculus (Fig. 1b and 1g top). Following unilateral disinhibitory injections of bicuculline into the superior colliculus a large increase in the baseline BOLD signal was recorded on the injected side, while a smaller response was observed on the non-injected side (Figs. 1d). After the immediate excitatory effects of the bicuculline began to dissipate (~1200-1880s Fig. 1d) clear light-evoked responses became evident (Figs. 1c, 1f and 1g bottom). Both the onset and offset of the light stimulus induced increases in the BOLD signal from the bicuculline injected superior colliculus (Fig. 1g bottom). A smaller potentiation of the light induced BOLD responses was recorded from the non-injected side. Disinhibition of superior colliculus also caused the region of light-evoked BOLD activity to extend ventrally into the collicular deep layers and to other regions targeted by this region, including the adjacent periaqueductal grey and underlying deep mesencephalic nucleus (Fig. 1c); and also the dorsal thalamus (not shown). Confirmation that these specific regions had been activated by a combination of the light stimulation and injection of bicuculline was provided by histological tissue processed for the activity marker c-fos; in this material the nuclei of activated cells contain black reaction product (compare Figs. 1c and 1e).

Conclusions

Using an established model of subcortical visual processing the present study has demonstrated that regional changes in neural responses to light stimuli evoked by local chemical stimulation of the midbrain superior colliculus can be measured with fMRI. These results indicate that fMRI is a viable technique for analyzing the circuit-wide consequences of direct local manipulations of neural activity on the brain's response to sensory stimulation.

References

Dommett E, Coizet V, Blaha CD, Martindale J, Lefebvre V, Walton N, Mayhew JE, Overton PG, Redgrave P. 2005. How visual stimuli activate dopaminergic neurons at short latency. Science 307(5714):1476-1479.