Functional imaging of brain activity in conscious monkeys responding to sexually arousing cues

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Olfactory cues can elicit intense emotional responses. This study used fMRI in male common marmoset monkeys to identify brain areas associated with sexual arousal in response to odors of ovulating female monkeys. Under light anesthesia, monkeys were secured in a specially designed restrainer and positioned in a 9.4 T magnetic resonance spectrometer. When fully conscious, they were presented with the scents of both ovariectomized and ovulating monkeys. The sexually arousing odors of the ovulating monkeys enhanced signal intensity in the preoptic area and anterior hypothalamus compared to the odors of ovariectomized monkeys. These data corroborate previous findings in monkeys based on invasive electrical lesion and stimulation techniques and demonstrate the feasibility of using non-invasive functional imaging on fully conscious common marmosets to study cue-elicited emotional responses. *NeuroReport* 12:2231–2236 © 2001 Lippincott Williams & Wilkins.

Key words: Anterior hypothalamus; BOLD; Common marmoset; Functional magnetic resonance imaging; Medial preoptic area; Pheromones; Sexual motivation

INTRODUCTION

Male sexual arousal can be evoked by a combination of different sexual stimuli in primates. In the common marmoset monkey (*Callithrix jacchus*), olfactory cues are important sexual stimuli. Male marmoset precopulatory behavior includes high rates of anogenital sniffing of sexually receptive females. Female marmosets deposit circumgenital scent marks that change with ovulatory status and males prefer scent marks from peri-ovulatory females over scent marks from non-ovulatory females [1]. Although the brain areas involved in processing the scent marks of sexually receptive females are unknown, lesioning the preoptic and anterior hypothalamic areas in male marmosets not only reduces male precopulatory and copulatory behavior [2] but also reduces male responsiveness to female proceptive sexual behavior [3]. These brain areas are likely to be involved in multiple aspects of male sexual behavior, possibly including the processing of sexually relevant olfactory cues. To test this hypothesis, we used fMRI in fully conscious marmoset monkeys. Increases in blood oxygen level-dependent (BOLD) signal [4–7] were measured in the preoptic area and anterior hypothalamus of male marmosets responding to scent mark odors of ovulatory, sexually receptive females and to ovariectomized (OVX) females. The data from this study show that fully conscious monkeys can be imaged while responding to ecologically relevant affective stimuli.

MATERIAL AND METHODS

MR studies were conducted on four adult male common marmoset monkeys that were family reared and socially housed. All subjects were extensively habituated to a specially designed MR restraint device and to the imaging procedure in a simulated environment. During MR sessions, animals were first lightly sedated with ketamine HCl (Ketaset, 2 mg) plus medetomidine (Domitor, 0.02 mg) and then placed in the MR head and body restraint. Once securely restrained, anesthesia was reversed with atipamezole (Antiseden, 0.1 mg). Animals were fully conscious within 12–30 min after anesthesia reversal. Anatomical imaging began ≥ 45 min after reversal, followed by functional imaging. A 40 mm diameter birdcage resonator was built into the head restraint and used in both transmit and receive modes. All images were acquired using a 9.4 T/

31 cm horizontal magnet (Magnex, Abingdon, UK) interfaced to a Unity INOVA console (Varian, Palo Alto, CA, USA) at the University of Minnesota. High-resolution anatomical data sets were acquired (fast spin echo, $TR = 2s$; echo train length = 8; echo train spacing = 9.5 ms; field of view = 4×4 cm; data matrix = 256×256; slice thickness = 2 mm) at the beginning and end of each imaging session. Functional images were acquired using a gradient-echo (FLASH) sequence ($TR = 115$ ms; $TE = 11$ ms; flip angle = 15°; field of view = 4×4 cm; data matrix = 128×128; slice thickness = 2 mm).

Female scent marks were collected and pooled from either peri-ovulatory or ovariectomized females, frozen at −70°C and thawed immediately prior to presentation. The fully conscious males were imaged during presentation of scent marks from peri-ovulatory and OVX females. BOLD fMRI data sets of seven slices each were collected continuously at 15 s intervals. Following 7 min of control data acquisition (28 seven-slice volume), peri-ovulatory and OVX scents applied to a wooden disc were positioned 9 cm from the nose of the monkey for 7 min followed by another 10 min of control data acquisition. Data collection was continuous over this 24 min period.

The boundaries delineating the region of interest (ROI) for the preoptic and anterior hypothalamic areas are shown in Fig. 2 and Fig. 3. Lloyd and Dixson [2], using electrolytic lesions, studied the potential role of the preoptic area and anterior hypothalamus in the control of sexual arousal in ten male common marmosets. The dimensions of each lesion were confirmed by histology and correlated to deficits in sexually relevant behaviors. The boundaries drawn for the ROIs in this study encompass the lesion dimensions published by Lloyd and Dixson. The anatomi-

cal images showing the ROIs were registered to comparable coronal images taken from the marmoset atlas of Saavedra and Massuchelli [8] and labeled accordingly. These ROIs were analyzed for changes in BOLD signal intensity using STIMULATE [9] software. Statistical comparison of control periods to olfactory stimulus periods was carried out using Student's paired *t*-test to generate an activation map for each control stimulus dataset. Those voxels from the original data whose percentage change [(average response over the stimulation period−average response over the control period)/(average response over the control period)] was identified as significant at a 95% confidence level were evaluated. Voxels satisfying these established criteria were overlaid on their respective anatomical data set.

RESULTS

Motion artifact was minimal in all of the imaging studies. Two to three anatomical data sets were collected over the course of each imaging study. Voxel-by-voxel subtractions of these data sets revealed no ostensible movement of the head between image acquisition protocols (Fig. 1), even though the monkeys were typically studied for >2 h in the MR spectrometer. Observations of movies comprising 96 slices for each of seven sections collected during 24 min of continuous functional imaging showed little motion artifact. Muscle movement associated with swallowing would occasionally affect image quality in a single slice.

Male marmosets tested with scent from a peri-ovulatory female showed a greater increase in BOLD signal in the preoptic (all of four monkeys; Table 1) and anterior hypothalamic (three of four monkeys, five of six sessions; Table 2) regions than when tested with OVX scent. The

Anatomical images acquired during study

A (t = 38 min)   B (t = 84 min)   C (t = 149 min)

Subtracted images

A − B   A − C
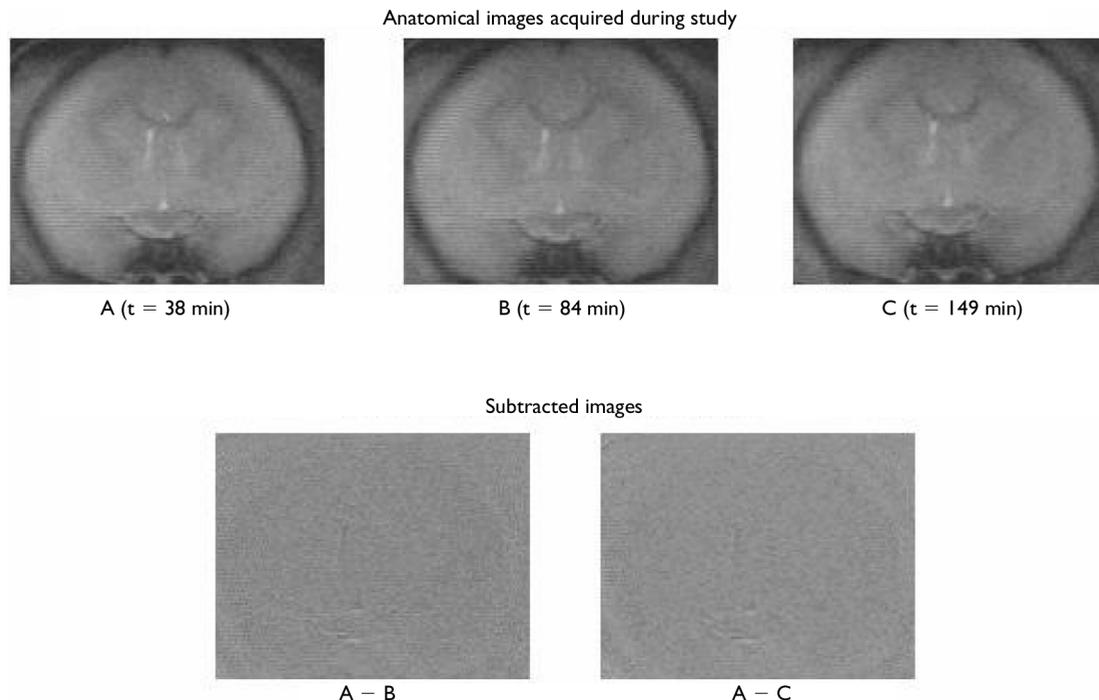
Fig. 1. Testing for motion artifacts. The top row shows a series of anatomical images taken at the time intervals noted. Images (**b**) and (**c**) were subtracted on pixel-by-pixel bases from the initial image (**a**) and appear in the bottom row.

Table 1. Enhanced BOLD signal in the preoptic area of male marmosets in response to scents from ovariectomized (OVX) and peri-ovulatory (Ovulate) females

Subject	Date	Voxels in ROI	No. voxels activated	
			OVX	Ovulate
CJ0097	10/21/00	174	2	5
CJ0555	10/22/00	163	11	19
CJ0413	10/22/00	164	3	7
CJ0527	10/21/00	154	1	9

Data are the number of voxels activated above baseline ($p < 0.05$) in the region of interest (ROI) for each subject and experimental date. The boundary of the ROI is shown in Fig 2.

Table 2. Enhanced BOLD signal in the anterior hypothalamic area of male marmosets in response to scents from ovariectomized (OVX) and peri-ovulatory (Ovulate) females

Subject	Date	Voxels in ROI	No. voxels activated	
			OVX	Ovulate
CJ0097	10/21/00	138	4	3
CJ0555	10/22/00	141	5	17
CJ0413	07/15/00	152	0	25
	10/22/00	163	4	12
CJ0527	07/15/00	152	2	5
	10/21/00	216	4	10

Data are the number of voxels activated above baseline ($p < 0.5$) in the region of interest (ROI) for each subject and experimental date. The boundary of the ROI is shown in Fig 3.

average percentage change in BOLD signal for the combined preoptic and anterior hypothalamic regions was 2–3% following OVX scent and 7–8% following peri-ovulatory scent. An example of enhanced BOLD activation in the preoptic area of one subject is shown in Fig. 2. Figure 3 shows enhanced activation in the anterior hypothalamus of another subject. Accompanying these activational maps are the respective time course data for each ROI (Fig. 2, Fig. 3). Average signal intensity is shown for the 30 sections collected prior to the presentation of scent (baseline) from a peri-ovulatory female. The response to the peri-ovulatory scent was almost immediate with an increase in average signal intensity that persists after withdrawal of scent for the duration of the data acquisition period. The response to OVX scent was also very rapid; however, in all cases the signal intensity appeared to diminish shortly after removal of scent.

DISCUSSION
In fully conscious male marmosets, olfactory cues from peri-ovulatory females selectively enhanced neuronal activity in the preoptic area and anterior hypothalamus. Data from many mammalian species describe a continuum of neurons extending from the preoptic area to the anterior hypothalamus area in the control of male sexual behavior. While most of these data come from studies on rodents, there are corroborating findings in monkeys. In male marmosets lesions of the preoptic–anterior hypothalamic continuum significantly reduced male sexual behavior [2]. The greatest disruption in sexual behavior occurs with

lesions at the level of anterior commissure (see Fig. 2). These lesions reduce precopulatory behavior such as: anogenital sniffing, tongue flicking and anticipatory penile erections as well as the frequency of mounting, intromission and ejaculation. These males showed normal positive social interactions with females attesting to the specificity of the brain damage to reduce sexual behavior but not general social activity. Furthermore, these lesioned males were less responsive to female proceptive behavior. Electrolytic lesions of these areas led to similar effects in rhesus macaques (*Macaca mulatta*) [10]. Electrical stimulation of the preoptic area and the dorsomedial hypothalamus in male rhesus monkeys elicited mounting behavior toward females [11]. In an operant paradigm used to assess the degree of sexual desire, male rhesus monkeys showed increases in neuronal electrical activity in the preoptic area in anticipation of sexual activity with receptive females [12].

Precopulatory tongue flicking and anticipatory penile erections in male marmosets do not require physical contact with the female; instead, these signs of heightened sexual arousal can be elicited by cues from receptive females. In these functional image studies, odors from receptive females were used to communicate with male marmosets during data acquisition in an MR spectrometer. The enhanced BOLD signal in the preoptic and anterior hypothalamic areas in response to ovulatory scent corroborates the previous findings noted above.

In these studies, the temporal resolution for scent-induced brain activation was rapid, occurring within 15 s
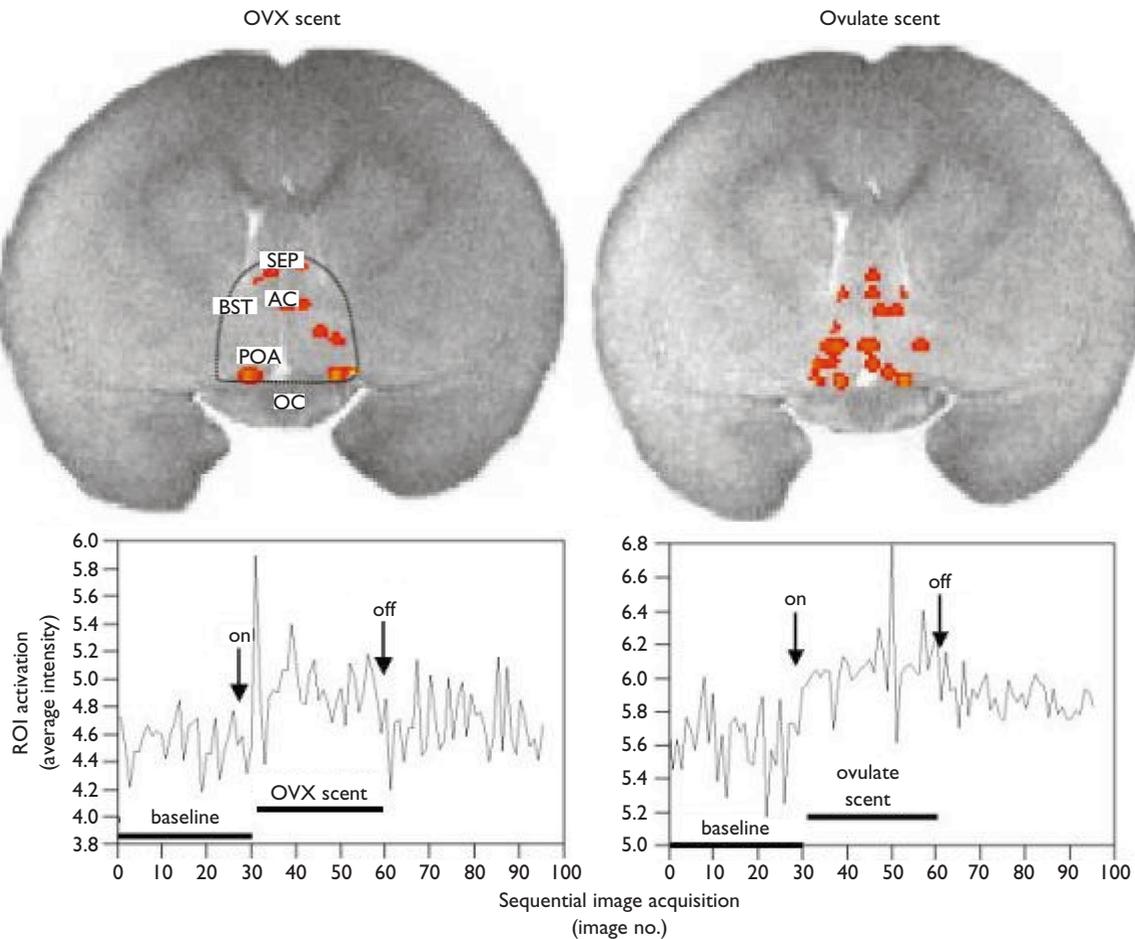
Fig. 2. Preoptic area activation. Scent from a peri-ovulatory female marmoset enhances BOLD signal in the preoptic area of male marmosets more than scent from an ovariectomized (OVX) female. The dome-shaped boundary shown in the left image outlines the region of interest (ROI). The average changes in signal intensity in response to OVX scent and ovulatory scent in the ROI are shown in the time course data below. SEP, septum; BST, bed nucleus of the stria terminalis; POA, preoptic area; OC, optic chiasm; AC, anterior commissure.

of stimulus presentation. This dynamic temporal pattern of odor-elicited brain activation with short or continuous exposure to a novel scent was also observed in the olfactory bulbs of anesthetized rats [13,14]. BOLD activation in the olfactory bulbs can be seen in < 8 s using a single slice acquisition protocol [14].

Until recently, conscious animals were not used in fMRI because of technical problems associated with movement of the animal in the MR spectrometer. Any minor head movement distorts the image and may also create a change in signal intensity that can be mistaken for stimulus-associated changes in brain activity [15]. In addition to head movement, motion outside the field of view can also obscure or mimic the signal from neuronal activation [16]. However, with a specially designed animal restrainer, evoked cortical activity in conscious rats was imaged in response to foot shock [17]. It would appear that BOLD signal changes maybe larger in conscious animals than in the anesthetized condition. For example, in studies using anesthetized animals, electrical stimulation of the rat's paw evokes changes in signal intensities in the somatosensory cortex of ~3% at 2.0 T [18]. In contrast, stimulation of the

rat's paw in an unanesthetized condition at 2.0 T evokes BOLD signal changes in the cortex ranging from 4 to 25% [17,19]. At 9.4 T forepaw stimulation in the anesthetized rat increases BOLD signal by almost 4% [20]. In the present study at 9.4 T, we report BOLD signal changes of up to 8% with olfactory stimulation in a fully conscious monkey. Lahti and co-workers [19] showed a clear distinction in BOLD signal intensity between the anesthetized and unanesthetized conditions by measuring evoked cortical activity in the same animal with intermittent anesthesia. The increased BOLD signal in the unanesthetized condition is most likely due to enhanced cerebral blood flow [21]. Functional MRI relies on changes in BOLD-based signal intensity that requires prompt and robust changes in local hemodynamics in response to increases in neuronal activity [4–7]. Because general anesthetics depress CNS metabolic activity and reduce basal cerebral blood flow [22] more robust BOLD signal changes are possible from conscious animals.

Conscious rhesus monkeys have been used to study stimulus-induced activation of the visual cortex. These studies were possible because rhesus monkeys can be
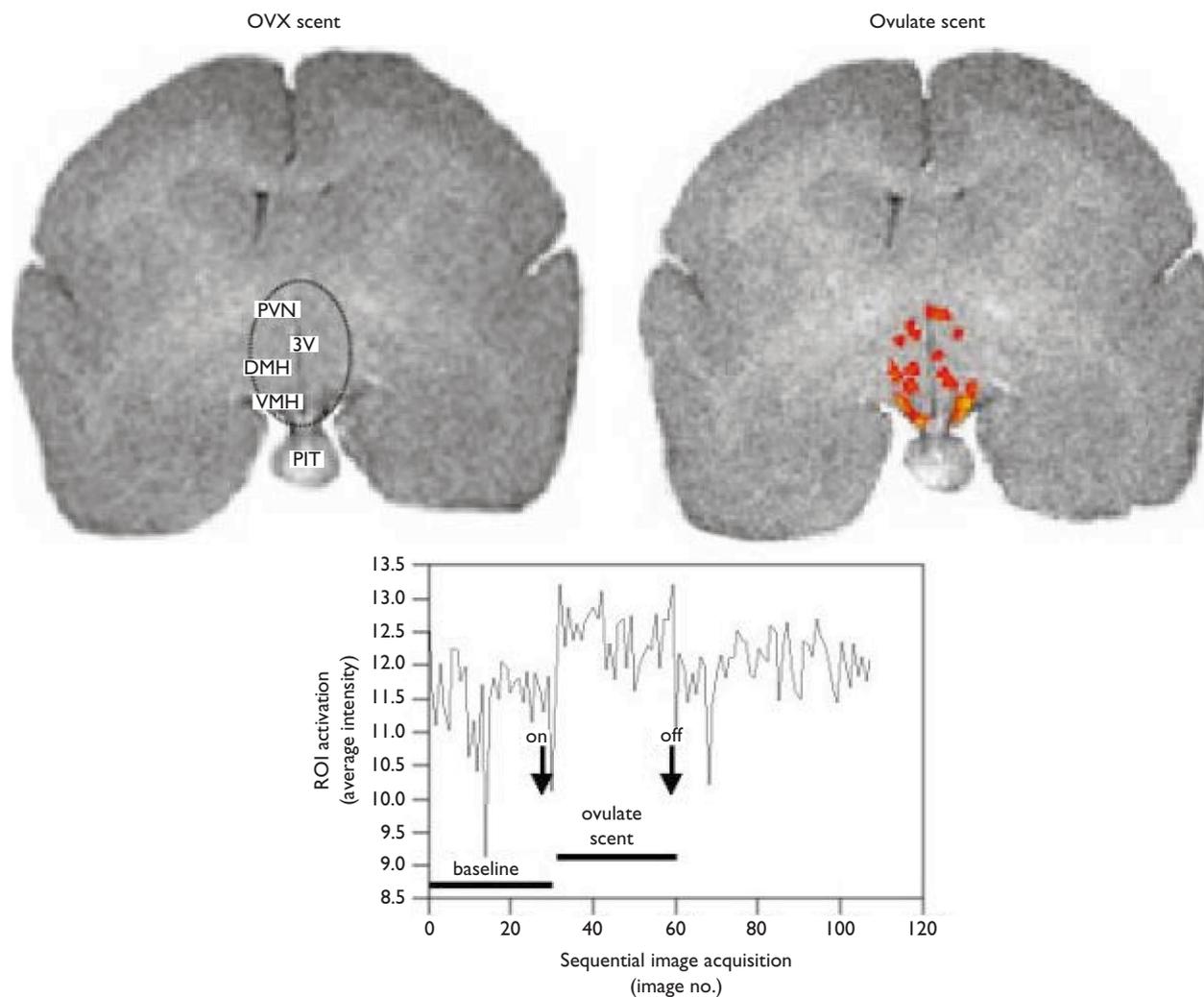
Fig. 3. Anterior hypothalamic area activation. Scent from a peri-ovulatory female marmoset enhances BOLD signal in the anterior hypothalamic region of male marmosets more than scent from an ovariectomized (OVX) female. The oval-shaped boundary shown in the left image outlines the region of interest (ROI). The average change in signal intensity in response to ovulatory scent in the ROI is shown in the time course data below. PVN, paraventricular nucleus; DMN, dorsomedial hypothalamus; VMH, ventromedial hypothalamus; 3V, third ventricle; PIT, pituitary gland.

trained to relax quietly in a sphinx-like position in a horizontal bore spectrometer [23] or to sit upright in a vertical bore spectrometer [24]. The visual stimuli in these studies carried minimal emotional valence that might elicit an autonomic response like fear or rage to increase the possibility of motion artifact.

CONCLUSION

The present study shows it is possible to perform fMRI on fully conscious monkeys using provocative stimuli that are ecologically relevant to elicit complex emotions like sexual arousal. Indeed, when using fMRI to study complex behaviors involving attention, motivation, emotion and cognition, anesthetized animals are unacceptable. The studies must be performed in the conscious preparations.

REFERENCES

1. Smith TE and Abbott DH. *Am J Primatol* **46**, 265–284 (1998).
2. Lloyd SAC and Dixson AF. *Brain Res* **463**, 317–329 (1988).
3. Dixson AF and Lloyd SAC. *Horm Behav* **23**, 211–220 (1989)
4. Ogawa S, Lee TM, Nayak AS *et al*. *Magn Reson Med* **14**, 68–78 (1990).
5. Ogawa S, Tank DW, Menon R *et al*. *Proc Natl Acad Sci USA* **89**, 5951–5955 (1992).
6. Kwong KK, Belliveau JW, Chesler DA *et al*. *Proc Natl Acad Sci USA* **89**, 5675–5679 (1992).
7. Bandettini PA, Wong EC, Hinks RS *et al*. *Magn Reson Med* **25**, 390–397 (1992).
8. Saavedra JP and Mazzucheli AL. *J Hirnforsch* **11**, 105–122 (1969).
9. Strupp JP. *Neuroimage* **3**, S607 (1996).
10. Slimp JC, Hart BL and Goy RW. *Brain Res* **142**, 105–122 (1978).
11. Perachio AA, Marr LD and Alexander M. *Brain Res* **177**, 127–144 (1979).
12. Oomura Y, Yoshimatsu H and Aou S. *Brain Res* **266**, 340–343 (1983).
13. Yang X, Renken R, Hyder F *et al*. *Proc Natl Acad Sci USA* **95**, 7715–7720 (1998).
14. Xu F, Kida I, Hyder F *et al*. *Proc Natl Acad Sci USA* **97**, 10601–10606

(2000).

15. Hajnal JV, Myers R, Oatridge A *et al. Magn Reson Med* **31**, 283–291 (1994).
16. Birm RM, Yetkin FZ and Hyde JS. *4th Scientific Meeting of the ISMRM Abstr* p. 1770 (1996).
17. Lahti KM, Ferris CF, Li F *et al. J Neurosci Methods* **82**, 75–83 (1998).
18. Scanley BE, Kennan RP, Cannan S *et al. Magn Reson Med* **37**, 969–972 (1997)
19. Lahti KM, Ferris CF, Li F *et al. Magn Reson Med* **41**, 412–416 (1999).
20. Silva AC, Lee S-P, Yang G *et al. J Cerebr Blood Flow Metab* **19**, 871–879

(1999).

21. Bonvento G, Charbonne R, Correze L *et al. Brain Res* **665**, 213–221 (1994).
22. Ueki M, Mies G and Hossmann KA. *Acta Anaesthesiol Scand* **36**, 318–322 (1992).
23. Dubowitz DJ, Chen D and Atkinson DJ *et al. Neuroreport* **9**, 2213–2218 (1998).
24. Logothetis NK, Guggenberger H, Peled S *et al. Nature Neurosci* **2**, 555–562 (1999).