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Imaging hypothalamic activity using diffusion weighted magnetic resonance imaging in the mouse and human brain ☆

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ABSTRACT

Hypothalamic appetite regulation is a vital homeostatic process underlying global energy balance in animals and humans, its disturbances resulting in feeding disorders with high morbidity and mortality. The objective evaluation of appetite remains difficult, very often restricted to indirect measurements of food intake and body weight. We report here, the direct, non-invasive visualization of hypothalamic activation by fasting using diffusion weighted magnetic resonance imaging, in the mouse brain as well as in a preliminary study in the human brain. The brain of fed or fasted mice or humans were imaged at 7 or 1.5 Tesla, respectively, by diffusion weighted magnetic resonance imaging using a complete range of b values ($10<b<2000$ s.mm^{-2}). The diffusion weighted image data sets were registered and analyzed pixel by pixel using a biexponential model of diffusion, or a model-free Linear Discriminant Analysis approach. Biexponential fittings revealed statistically significant increases in the slow diffusion parameters of the model, consistent with a neurocellular swelling response in the fasted hypothalamus. Increased resolution approaches allowed the detection of increases in the diffusion parameters within the Arcuate Nucleus, Ventromedial Nucleus and Dorsomedial Nucleus. Independently, Linear Discriminant Analysis was able to classify successfully the diffusion data sets from mice and humans between fed and fasted states. Present results are consistent with increased glutamatergic neurotransmission during orexigenic firing, a process resulting in increased ionic accumulation and concomitant osmotic neurocellular swelling. This swelling response is spatially extendable through surrounding astrocytic networks until it becomes MRI detectable. Present findings open new avenues for the direct, non-invasive, evaluation of appetite disorders and other hypothalamic pathologies helping potentially in the development of the corresponding therapies.

Introduction

The appetite impulse originates in the brain from an imbalance in the systemic and intrahypothalamic mechanisms controlling food intake and energy expenditure (Morton et al., 2006). Following a meal, increased insulin and leptin levels induce an anorexigenic response consisting in a reduction in food intake and an increase in energy expenditure, whereas in fasting periods decreased plasma levels of leptin and insulin promote increased food intake and energy expenditure. These systemic and intrahypothalamic neuroendocrine signals interact mainly in the Arcuate Nucleus (ARC), the Ventromedial Nucleus (VMN), Dorsomedial Nucleus (DMN) and the Paraventricular Nucleus (PVN), modifying the balance between the activity of orexigenic neurons, releasing Agouti Related Peptide (AgRP) or Neuropeptide Y (NPY), and anorexigenic neurons releasing Proomileanocortin (POMPCT) or Cocaine and Amphetamine Regulated Transcript (CART) (Coll et al., 2007). In addition to neuroendocrine signaling, appetite stimulation is known to involve hypothalamic increases in glutamatergic and gabaergic neurotransmissions (Delgado et al., 2011; van den Pol et al., 1990). Moreover, it has been recently reported (Spanswick et al., 2012) that the excitatory synaptic control of AgRP neurons is regulated by fasting and hormones, and that increased glutamatergic activity is a necessary requirement for a physiological response to fasting (Liu et al., 2012; Yang et al., 2011).

Despite the progress obtained in the interpretation of the molecular events underlying the fasting response, the direct evaluation of appetite regulation in the brain still remains difficult, very often limited to

Abbreviations: D_{slow}, Slow diffusion coefficient; D_{fast}, Fast diffusion coefficient; FDP, Fast diffusion phase; LDA, Linear Discriminant Analysis; SDP, Slow diffusion phase.

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indirect measurements of food intake and body weight. On these grounds, the implementation of non-invasive methodologies for the evaluation of appetite entails considerable relevance. A variety of neuroimaging tools have been proposed including mainly positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) methods (Carnell et al., 2011). PET studies provide information on cerebral activation by detecting the emitted positrons derived from the increased uptake of ^{18}F-deoxiglucose, an event revealing the metabolic coupling between glucose uptake, blood flow and neuronal activity in the hypothalamus during feeding-related stimuli (Gautier et al., 2000; Tataranni et al., 1999). Blood Oxygenation Level Dependent (BOLD) fMRI, infers regional neuronal activity from the changes in magnetic susceptibility that take place during the transition between oxygenated hemoglobin and deoxygenated hemoglobin, occurring after increased oxygen delivery to activated neurons in the hypothalamus of rats and humans (Mahankali et al., 2000; Matsuda et al., 1999). Finally, manganese enhanced magnetic resonance imaging (MEMRI), uses manganese ion accumulation as a surrogate marker of the increased calcium movements occurring during neuronal activation. MEMRI has revealed the time course of hypothalamic activation as a response to the systemic administration of different orexigenic or anorexigenic peptides (Parkinson et al., 2009). Notably, these previous approaches are not devoid of limitations to investigate hypothalamic physiology in animals and man, mainly derived from their reduced spatial and temporal resolution in the PET and BOLD fMRI approaches, and the potential neurotoxicity of Mn^{2+}, in the MEMRI technique. To overcome these limitations, we propose here the use of functional diffusion weighted imaging fDWI (Le Bihan, 2003) a novel functional approach improving the spatial and temporal resolution provided earlier by PET or BOLD and avoiding the use of the potentially toxic doses of manganese precluding the use of MEMRI in humans.

Materials and methods

Experimental models

The experimental protocols used in this study were approved by the appropriate institutional committees and met the guidelines of the appropriate government agency. Experiments with animals were carried out using healthy adult male C57BL/6 mice (n = 12) aged nine weeks. Each animal was investigated in two successive experimental conditions both receiving drinking water ad libitum; "fed"; receiving normal mice chow diet (A04 http://www.safe-diets.com/eng/home/home.html, SAFE Augy, France, 2900 kcal/kg), and "fasted"; following either 48 h (group 1, n = 6, 25 ± 4 g) or 16 h (group 2, n = 6, 26 ± 2 g) after complete food removal. In all small animal imaging experiments, anesthesia was initiated in a plexiglass induction box (Isofluorane 2%/99.9% O2, 1 mL/min) and maintained during the imaging time with a nose mask (Isofluorane 1%/O2 99.9% mixture, 1 mL/min). Anesthetized animals were placed in a water heated probe, which maintained the core body temperature at approximately 37 °C during scanning. The physiological state of the animal during the imaging process was monitored by the respiratory rate and body temperature using a Biotrig physiological monitor (Bruker Biospin, Ettlingen, Germany).

We performed a pilot study with human subjects to illustrate the potentialities of the proposed methodology in a routine clinical environment. Human participants in the study were six healthy male volunteers, aged 24–33. Conditions for the participation were: (1) healthy clinical trajectory without familiar history of obesity, diabetes or other endocrine disorders; (2) Body Mass Index (BMI) of 18.5–25, corresponding to normal body weight; and (3) volunteers were required to follow a balanced diet (2000–2500 cal/kg) during seven days prior to the basal image acquisitions, with no drinks other than water ad libitum, no medication or abnormal exercise. Specific instructions to follow a balanced diet were provided for each individual at the beginning of the study and the degree of compliance with these obtained individually before the imaging sessions. All individuals adhered correctly to the outlined protocol. Each volunteer was imaged in two successive conditions; first, "fed", after one week of a balanced diet and second "fasted", 24 h after food deprivation. Blood samples from the median cubital vein were drained before the "fed" and "fasted" image acquisitions and analyzed for routine biochemical parameters T3, T4, TSH and insulin levels.

MRI sequences

The magnetic resonance imaging (MRI) experiments with mice were performed on a 7.0-T horizontal-bore (16 cm) superconducting magnet equipped with a ^{1}H selective birdcage resonator of 23 mm and a 90 mm diameter gradient insert (36 gauss/cm). Imaging data were acquired using Hewlett-Packard console running Paravision 4.0 software (Bruker Medical Gmbh, Ettlingen, Germany) operating on a Linux environment.

Fig. 1 provides an overview of the acquisition and image analysis approaches implemented in this study and the regions of interest investigated, as illustrated for the mouse brain. A collection of diffusion weighted images of the fed and fasted mouse brain was obtained (left panel) and analyzed using either a biexponential diffusion model (upper right panels) or a model-independent Linear Discriminant Analysis approach (lower right panels). The set of DWI was acquired with the conditions indicated below, across an axial plane containing the hypothalamus (Paxinos and Franklin, 2001) with the diffusion gradient oriented along three orthogonal directions; Left–Right (L–R), Antero-Posterior (A–P) and Head–Feet (H–F). This structure was localized using a sagittal section showing the pituitary gland and selecting after the first axial section rostral to it, as indicated in Fig. 2A. This section is located anatomically Bregma −1.46 mm. In the six mice of group 1, the acquisition parameters were (Bruker Pharmascan, Bruker Medical Gmbh, Ettlingen, Germany): repetition time (TR) = 3000 ms, echo time (TE) = 51 ms, four shot EPI readout, averages (Av) = 3, Δ = 20 ms, δ = 4 ms, field of view (FOV) = 35 mm, acquisition matrix = 128 × 128, corresponding to an in-plane resolution of 296 × 296 μm^2, slice thickness of 1.5 mm, number of slices = 3, using a collection of five low b values ($10<b<100$ s.mm^{-2}) and six high b values ($200<b<1800$ s.mm^{-2}).

The six mice of group 2, were investigated using nine diffusion weighted images (DWI) acquired along the axial plane containing the hypothalamus, with b values ($300<b<2000$ s.mm^{-2}). Acquisition parameters were (Bruker AVANCE III, Bruker Medical Gmbh, Ettlingen, Germany): repetition time (TR) = 3000 ms, echo time (TE) = 31 ms, four shot EPI readout, averages (Av) = 3, Δ = 20 ms, δ = 4 ms, field of view (FOV) = 21 mm, acquisition matrix = 128 × 128, corresponding to an in-plane resolution of 164 × 164 μm^2, slice thickness of 1.25 mm and number of slices = 3.

T_2-weighted (T_{2w}) spin echo anatomical images were acquired previous to the DWI with improved resolution to localize and resolve precisely the hypothalamic region in every mouse. Axial T_{2w} images across the section containing the hypothalamus were acquired using the rapid acquisition with relaxation enhancement (RARE) sequence with the following parameters: TR = 3200 ms, TE = 60 ms, RARE factor = 8, Av = 3, FOV = 38 mm (Pharmascan console), 21 mm (AVANCE III console), acquisition matrix = 256 × 256 corresponding to an in-plane resolution of 148 × 148 μm^2 (Pharmascan console) or 80 × 80 μm^2 (AVANCE III console), number of slices = 3 and slice thickness = 1.5 mm (Pharmascan console), or 1.25 mm (AVANCE III console).

The magnetic resonance imaging (MRI) experiments with human volunteers were performed in the Magnetic Resonance Unit of the Hospital Nuestra Señora del Rosario (Madrid, Spain), using a GE Medical Systems 1.5-T horizontal-bore superconducting magnet, equipped with a ^{1}H quadrature head resonator. Prior to the imaging experiments, volunteers signed up an informed consent and confidentiality document. Image acquisitions were medically supervised by the neuroradiology