NeuroImage 83 (2013) 294–306

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

MRI-based morphometric characterizations of sexual dimorphism of the cerebrum of ferrets (*Mustela putorius*)

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ARTICLE INFO

Article history:
Accepted 3 June 2013
Available online 14 June 2013

Keywords:
Phylogeny
Evolution
Brain
Neurodevelopmental disorders
Sexual dimorphism

ABSTRACT

The present study aimed to characterize cerebral morphology in young adult ferrets and its sexual dimorphism using high-field MRI and MRI-based morphometry. *Ex vivo* short TR/TE (typical T_1-weighted parameter setting for conventional MRI) and T_2W (long TR/TE) MRI with high spatial resolution at 7-tesla could visualize major subcortical and archicortical structures, i.e., the caudate nucleus, lentiform nucleus, amygdala and hippocampus. In particular, laminar organization of the olfactory bulb was identifiable by short TR/TE-MRI. The primary and secondary sulci observable in the adult ferret were distinguishable on either short TR/TE- or T_2W-MRI, and the cortical surface morphology was reproduced well by 3D-rendered images obtained by short TR/TE-MRI. The cerebrum had a significantly lower volume in females than in males, which was attributed to region-specific volume reduction in the cerebral cortex and subcortical white matter in females. A sexual difference was also detected, manifested by an overall reduction in normalized signal ratios of short TR/TE-MRI in all cerebral structures examined in females than in males. On the other hand, an alternating array of higher and lower short TR/TE-MRI intensity transverse zones throughout the cortex, which was reminiscent of the functional cortical areas, was revealed by maximum intensity projection (MIP) in 3D. The normalized signal ratio of short TR/TE-MRI, but not T_2W-MRI in the cortex, was negatively correlated with the density of myelin-basic protein immunoreactive fibers (males, $r = -0.440$; females, $r = -0.481$). The present results suggest that sexual differences in the adult ferret cerebrum are characterized by reduced volumes of the cerebral cortex and subcortical white matter in females, and by overall reductions in physiochemical characteristics, as obtained by short TR/TE-MRI, in females. It should be noted that short TR/TE-MRI-based MIP delineated functional cortical areas related to myeloarchitecture in 3D. Such an approach makes possible conventional investigation of the functional organization of the cerebral cortex and its abnormalities using high-field MRI.

Abbreviations: A1, primary auditory cortex; aad, anterior ancinate dimple; AAF, anterior auditory field; ac, anterior commissure; aci, intrabulbar anterior commissure; ADF, anterior dorsal field; as, ancinate sulcus; AEG, anterior ectosylvian gyrus; ASG, anterior sigmoid gyrus; Amg, amygdala; AVF, anterior ventral field; cc, corpus callosum; Cd, caudate nucleus; cg, cingulum; CNG, coronal gyrus; cns, coronal sulcus; crs, cruciate sulcus; csss, caudal suprasylvian sulcus; ec, external capsule; EPI, external plexiform layer; Gl, glomerular layer; Hip, hippocampus; ic, internal capsule; IPI, internal plexiform layers; LG, lateral gyrus; Ln, lentiform nucleus; ls, lateral sulcus; M1, primary motor cortex; MBP, myelin basic protein; Mi, mitral layer; MIP, maximum intensity projection; Ob, olfactory bulb; Obc, olfactory bulb core; OBG, orbital gyrus; OD, optical density; opr, optic radiation; ots, occipitotemporal sulcus; oSVZ, outer subventricular zone; PEG, posterior ectosylvian gyrus; PFA, paraformaldehyde; PPr, rostral posterior parietal cortex; prs, presylvian sulcus; PPF, posterior pseudosylvian field; prs, presylvian sulcus; PSF, posterior suprasylvian field; PSG, posterior sigmoid gyrus; pss, pseudosylvian sulcus; rf, rhinal fissure; rs, rhinal sulcus; rsss, rostral suprasylvian sulcus; spt, septum; S1, primary somatosensory cortex; SII, secondary somatosensory cortex; SIII, third somatosensory cortex; SSG, suprasylvian gyrus; subWM, subcortical matter; ss, splenial sulcus; Th, thalamus; VCA, visual cortical area.

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http://dx.doi.org/10.1016/j.neuroimage.2013.06.024

Introduction

The remarkable development and spread of imaging techniques such as MRI and CT in recent years have provided full 3D brain coverage, while leaving the gross anatomy conserved. Quantitative analysis methods using these imaging techniques allow the investigation of focal differences in brain structures between a control and experimental group, or time-dependent changes in brain structures within groups (Wright et al., 1995). A number of studies have been attempted to explain structural sexual dimorphism of brain regions, quantitatively. Sex differences are noted in volumes of particular regions of the cerebral cortex, hippocampus, caudate nucleus, globus pallidus and/or olfactory bulb in humans (Carne et al., 2006; Giedd et al., 1997; Suzuki et al., 2005) and mice (Schlaepfer et al., 1995; Spring et al., 2007). Several studies revealed that sex-related volume changes in specific regions of the brain are involved in neurodevelopmental and psychological disorders such as schizophrenia (Exner et al., 2008), amnestic mild cognitive impairment, Alzheimer's disease

(Skup et al., 2011), and panic disorder (Asami et al., 2009) in humans. In addition, a higher prevalence of autism is found in males than in females, and appears to show abnormalities in the cerebral cortical surface morphology (Harden et al., 2004; Levitt et al., 2003). Therefore, quantitative data regarding sexual dimorphism of various regions provide fundamental information for investigating the pathogenesis of sex-related neurodevelopmental and psychological disorders.

Ferrets (*Mustela putorius*) are the smallest laboratory animals that have a highly convoluted surface of the cerebrum, forming striking patterns of sulci and gyri (Lawes and Andrews, 1998). This animal has advantages as an experimental model for studying the plasticity and development of the central nervous system. Neurodevelopmental events occurring *in utero* in primates, such as the early stage of neurogenesis (Clancy et al., 2001), sulcal infolding (Sawada and Watanabe, 2012; Smart and McSherry, 1986), and the transient appearance of the outer subventricular zone (oSVZ) with basal radial glia (Fietz et al., 2010; Martínez-Cerdeño et al., 2012), are experienced in ferrets following birth. This means that experimental magnifications such as drug administration and stress loading can be applied directly to the pups. Furthermore, ferrets are used as an animal model for a debilitating respiratory disease, human influenza, because of their high sensitivity to infection by human influenza viruses and diseases closely resembling human influenza (Maher and DeStefano, 2004). This means that ferrets have the potential for application to studies of influenza-associated encephalopathy. The present study aimed to characterize the cerebral morphology in young adult ferrets and its sexual dimorphism using MRI-based morphometry. To achieve a sufficient resolution for determining individual structures, fixed forebrain was examined using a high spatial resolution 7-tesla MRI system.

Materials and methods

Samples

The present study used cerebra from male and female ferrets at postnatal day (PD) 90 (male, n = 5; female, n = 5) that had been previously used in our gross anatomical examination of cerebral surface morphology (Sawada and Watanabe, 2012). Briefly, the animals were deeply anesthetized with an intraperitoneal injection of chloral hydrate (400 μg/g body weight), and were perfused with 0.9% NaCl followed by 4% paraformaldehyde (PFA) in a 10 mM phosphate buffer, pH 7.4. Brains were removed from skulls, and immersed in the same fixative.

MRI measurements

Three-dimensional T_1-weighted (short TR/TE) and two-dimensional T_2W (long TR/TE) MRI were performed with a 7.0-T MRI system (Magnet; Kobelco and Jastec, Kobe, Japan) (Console; Bruker BioSpin, Ettlingen, Germany). A birdcage RF coil for transmission and reception (70-mm inner diameter, Rapid Biomedica; or 60-mm inner diameter, Bruker BioSpin) was used with a field of view adequate for the sample dimensions. Slice orientation (transaxial) was precisely adjusted for the cerebral base using pilot-MR images obtained by gradient-echo sequence. A three-dimensional T_1-weighted image covering the entire brain was acquired using the rapid acquisition with relaxation enhancement (RARE) sequence, with the following parameters: repetition time (TR) = 300 ms, echo time (TE) = 9.6 ms (effective TE = 19.2 ms), RARE factor = 4, field of view (FOV) = 32 × 32 × 40 mm^3, acquisition matrix = 256 × 256 × 256, voxel size = 125 × 125 × 156 μm^3, number of acquisitions (NEX) = 2, and total scan time = 2 h 43 min 50 s. A multi-slice T_2-weighted image covering the entire brain was acquired using the RARE sequence with the following parameters: repetition time TR = 4200 ms, TE = 12 ms (effective TE = 36 ms), rare factor = 8, FOV = 38.4 × 38.4 mm^2, slice thickness = 1.0 mm, slice gap = 1.5 mm, number of slices = 10, acquisition matrix = 256 × 192 (reconstructed to 256 × 256 using zero-filling method), voxel size = 150 × 150 × 1000 μm^3, NEX = 40, and total scan time = 1 h 7 min 12 s.

We used the term "short TR/TE MRI" in the context of "MRI measured using typical T_1W parameter setting" in the present study, because the T_1W parameter setting did not show T_1-weighted contrast at the PFA-fixed brain sample under high-field MRI. Although shorter T_1 tissue, such as white matter, must be enhanced on T_1W-MRI, the signal was reduced due to the short T_2 of PFA solution (Supplement 1).

3D volume-rendered images

All 3D data were used for volumetric analysis. The cerebral cortex, olfactory bulb, caudate nucleus, lentiform nucleus (putamen plus globus pallidus), amygdala and hippocampus were semi-automatically segmented on MRI images using the "Morpho" tool of SliceOmatic software ver 4.3 (TomoVision, Montreal, Canada) based on image contrast as well as the user's knowledge of the anatomy. Segmented images were then analyzed using the 3D-rendering module of the same software. The cerebral image was rendered in 3D using the surface projection algorithm which best visualized the surface and sulci of the cerebrum. The 3D-rendered images were then rotated and manipulated in a manner that best visualized brain morphology by a linear registration method using SliceOmatic software. A clear indentation at the cerebral surface was considered the indication of a sulcus. A gyrus was defined as any tissue delimited by two or more fissures, sulci, or dimples. The terminology and identification of cerebral sulci and gyri were based on the textbook of Lawes and Andrews (1998).

Volumetric analysis

Areas of the segmented regions of each cerebral region were measured using SliceOmatic software. The volumes were calculated by multiplying the combined areas by the slice thickness (156.25 μm) with the total areas of those regions being regarded as the volume of the whole cerebrum. Data on the volume of each cerebral structure of the left and right hemispheres were analyzed separately.

Data analysis of short TR/TE and T_2W-MRI

Signal levels of short TR/TE and T_2W-MRI intensity were quantified by specifying the region of interest (ROI) in the known cortical areas, and in the major subcortical and archicortical structures. The following cortical regions were selected on the maximum intensity projection (MIP) color map by registration using the "Point" tool of SliceOmatic software for specifying the ROI, and signal intensities in each ROI: the primary motor cortex (M1; the rostral halves of posterior sigmoid gyrus (PSG) and coronal gyrus (CNG)), secondary somatosensory cortex (SII; the rostral half of the anterior ectosylvian gyrus (AEG)), primary auditory cortex (A1; the upper half of posterior ectosylvian gyrus (PEG)) as the cortical areas exhibiting a relatively low short TR/TE intensity, primary somatosensory cortex (S1; the caudal halves of PSG and CNG), rostral posterior parietal cortex (PPr; the rostral half of lateral gyrus (LG)), and area 17 (the caudal half of the visual cortical area (VCA)) as the cortical areas exhibiting a relatively high short TR/TE-MRI intensity. Intensities of short TR/TE- and T_2W-MRI were measured as background signals in the medium (4% PFA solution) that filled the brains. We defined the ROIs for the background signal at the points nearest (within 2–10 mm apart on the same coronal images) of ROIs for each brain region, in order to minimize the effect of RF inhomogeneity. Typical localizations of ROIs in each brain region and the respective background signals are shown in Supplement 2. The normalized signal ratios of short TR/TE and T_2W-MRI intensity were calculated by a formula [(signal intensity in the brain region) / (signal intensity of the nearest medium)]. Here, we carefully assessed the RF coil inhomogeneity (B_1 profile;