Sex steroid hormones-related structural plasticity in the human hypothalamus.

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Abstract: We investigated the effects of an artificial menstrual cycle on brain structure and activity in young women using metabolic magnetic resonance imaging (MRI). We show that the activation of the hypothalamo-pituitary-gonadal axis during the pill-free interval of low dose combined oral contraceptive use is associated with transient microstructural and metabolic changes in the female hypothalamus but not in the thalamus, a brain structure unrelated to reproductive control, as assessed by water diffusion and proton magnetic resonance spectra measurements. Our results provide neuroanatomical insights into the mechanism by which sex steroid hormones mediate their central effects and raise the intriguing possibility that specific regions of the neuroendocrine brain use ovarian cycle-dependent plasticity to control reproduction in humans. These MRI-based physiological studies may pave the way for the development of new diagnostic and treatment strategies in the central loss of reproductive competence in human syndromes, such as hypothalamic amenorrhea.

1. Cover Letter

novembre 24, 2009

Dr Katrin Amunts

Section Editor,
NeuroImage

Dear Dr. Amunts,

Please find enclosed the revised version of our manuscript ID NIMG-09-680R1 entitled “Sex steroid hormones-related structural plasticity in the human hypothalamus”, which we have revised to address the points raised by the reviewer 2 as follows:

Reviewer 2:

1) We apology for this omission. Page 7, line 10, it now reads “voxel size 10x10x10 mm^3”

2) Page 7, lines 1-3, it now reads “Inclusion of little amounts of CSF could have occurred during the placement of the ROI within the hypothalamus. However, the putative inclusion of CSF in the Hypothalamic ROIs was random and thus very unlikely to influence the results.”

We thank once again the referees for their thoughtful comments and hope that the manuscript is now in suitable form for publication in NeuroImage.

Sincerely,

Vincent Prevot, Ph.D.
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59045 Lille cedex
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***2. Response to Reviews**

Reviewer 2:

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Submission: November 24, 2009
NeuroImage

Sex steroid hormones-related structural plasticity in the human hypothalamus

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***** MB and PJ contributed equally to the work.

Abbreviated title: Brain plasticity and artificial menstrual cycle

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Abstract

We investigated the effects of an artificial menstrual cycle on brain structure and activity in young women using metabolic magnetic resonance imaging (MRI). We show that the activation of the hypothalamo-pituitary-gonadal axis during the pill-free interval of low dose combined oral contraceptive use is associated with transient microstructural and metabolic changes in the female hypothalamus but not in the thalamus, a brain structure unrelated to reproductive control, as assessed by water diffusion and proton magnetic resonance spectra measurements. Our results provide neuroanatomical insights into the mechanism by which sex steroid hormones mediate their central effects and raise the intriguing possibility that specific regions of the neuroendocrine brain use ovarian cycle-dependent plasticity to control reproduction in humans. These MRI-based physiological studies may pave the way for the development of new diagnostic and treatment strategies in the central loss of reproductive competence in human syndromes, such as hypothalamic amenorrhea.

Key words: Diffusion, Spectroscopy, MRI, Hypothalamus, Ovarian cycle, Plasticity

Introduction

Rodent studies have shown that the hypothalamus is a useful brain structure for the study of hormone- and activity-dependent plasticity both during development (Simerly, 2005) and in adults (Hatton, 1997; Prevot et al., 2007; Theodosis et al., 2008). Current models of neuronal plasticity greatly stress the importance of transient electrical and biochemical events associated with the excitation process. However, there is compelling evidence that activation is accompanied by other important physical phenomena. Microstructural changes in tissues have been observed in response to the changing physiological context, performed initially in electron microscopy studies (Hatton, 1997; Theodosis et al., 2008) and subsequently through in vitro diffusion measurements (Piet et al., 2004). These studies have revealed, for instance, that in discrete hypothalamic nuclei, astrocyte retraction is one of the physiological responses associated with neuronal activation during lactation (Oliet et al., 2001; Panatier et al., 2006). Similarly, it has been established that fluctuating levels of gonadal steroids during the ovarian cycle have the capacity to alter the astroglial sheath, thus regulating "neuroendocrine synapse" formation by hypothalamic neurons that release gonadotropin releasing hormone (GnRH) (King and Letourneau, 1994; Prevot et al., 1999; Yamamura et al., 2004; de Seranno et al., 2010), the neuropeptide that controls gonadotropin secretion and reproduction. However, such plastic events have been monitored using fixed tissues or invasive techniques in brain slices, which may not fully reflect physiological conditions. Non-invasive longitudinal studies monitoring hormone-controlled plasticity in animals or humans would thus have a tremendous impact, directly linking neural events, such as membrane retraction, to the underlying homeostatic changes.

In women, the hypothalamic-pituitary responses to gonadal steroid feedback during the ovarian cycle are both dose and time dependent (Hotchkiss and Knobil, 1994). Acute or chronic exposure to low concentrations of gonadal steroids inhibits gonadotropin secretion (Taylor et al., 1995), whereas a progressive increase in estrogen over a period of several days stimulates luteinizing hormone (LH) secretion (Liu and Yen, 1983; Taylor et al., 1995).

How these contrasting effects are mediated and whether they involve structural plasticity within the hypothalamus remains unknown. With the development of advanced magnetic resonance imaging (MRI) techniques such as diffusion and spectroscopy, tissue structure can now be probed and imaged on a microscopic scale *in vivo* (Ross and Bluml, 2001; Le Bihan, 2003). The data collected using these techniques provide unique clues to the fine architecture of neural tissues, the metabolic activity of discrete brain areas, and changes associated to various physiological states. Taking advantage of these advanced imaging techniques we tested the hypothesis that variation in gonadal steroid levels during an artificial menstrual cycle in which timing and nature of the changes in gonadotropic secretion are well characterized (Hemrika et al., 1993), evoke changes in morphological homeostasis within the hypothalamus of the human brain.

To address this question two different metabolic MRI approaches were used that have, in contrast to functional MRI (Logothetis, 2008), spatial resolution compatible with the study of restricted brain structures such as the hypothalamus. One approach consisted of assessing the water diffusion coefficient, which provides information on the microscopic obstacles that hinder diffusing molecules, such as membranes and macromolecules, thus providing information about tissue cellular structure (Le Bihan, 2003). The other technique was proton MR spectroscopy, which allows for measurement of a range of cerebral metabolites, such as the neuronal marker N-acetyl-aspartate (NAA) and, choline (Cho) and creatine (Cr), compounds that are present in all neural cell types but appear to be at much higher concentrations in astroglia than in neurons (Urenjak et al., 1993). Female volunteers were subjected to MRI at two stages of their artificial menstrual cycle: 13 days (d13) after initiating oral contraception when the hypothalamic-pituitary-gonadal axis is fully inhibited (Hemrika et al., 1993) and at the end of the pill-free interval (d-2) when most of the steroidogenic negative feedback effects wear off and a normal early follicular phase pulsatile LH pattern is found (Hemrika et al., 1993). Here we report that removal of the oral contraceptive-mediated gonadal steroid negative feedback on the reproductive axes dramatically and selectively favors diffusion in the hypothalamus and is associated with a decrease of the choline/NAA

ratio. The results are interpreted in terms of structural changes within the hypothalamus in inhibitory-gonadal steroids-deprived conditions and links with the homeostatic changes accompanying hypothalamic activity are discussed.

Materials and Methods

Subjects

The study was done with the approval of the local ethical committee and informed consent was obtained from each subject. The study included 20 healthy volunteers (10 men and 10 women), according to a standard medical examination, i.e, no sign of neurological deficit and no alteration of the physical well-being. Their age (from 19 to 25 years) and body mass index (BMI, of 18 to 24) distributions were uniform (t-test, $p > 0.05$). Male volunteers received no treatment. All women had a history of regular menses (24-35 days) before starting oral contraception and had been using low dose combined oral contraceptive (mono, bi or triphasic pills containing from 20 to 40 µg of ethinylestradiol and from 50 to 300 µg of progestins for 21 days out of a 28-d cycle) for at least 3 months prior to entering the study. When beginning the treatment, all the women started their pills the first day of menses. Oral contraceptives inhibit folliculogenesis by a central suppressive action on the release of gonadotropins; the kinetic of their effects on the human hypothalamic pituitary gonadal axes is well characterized (Hemrika et al., 1993). Under a low dose combined oral contraceptive, FSH levels are rapidly suppressed (from day 2) and LH levels decline from day 8. After the 7-day pill-free interval, a normal early follicular phase pulse pattern of LH is found, even in long term oral contraceptives users (Hemrika et al., 1993). All subjects had eaten a standard breakfast before being subjected to magnetic resonance imaging (MRI) for total scan duration of 30 min (15 min for diffusion MRI and 15 min for spectroscopy).

Conventional MRI

The magnetic resonance (MR) examination was made with a 1.5T scanner (ACHIEVA R1.5.1, Philips, Best, The Netherlands) using a SENSE eight-channel head coil. All sequences (including Diffusion) were designed to cover the entire thalamus and hypothalamus. The anatomical limits were, the genu of the corpus callosum at the anterior to the posterior aspect of the callosal splenium at the posterior. The protocol included 3D Fast Field Echo T1-weighted (TR/TE: 25/4.6 ms; Nex: 2; Flip angle: 30; FOV: 230/183/60; Matrix: 272x216; 75 slices; Voxel size: 0.85/0.85/0.80), Inversion-recovery T1-weighted (TR/TI/TE: 7910/400/15 ms; Nex: 2; FOV: 230/195/73; Matrix: 512x261; 20 slices; Slice thickness: 3 mm; Slice gap: 0.7 mm), Turbo Spin Echo T2-weighted (TR/TE: 3500/100 ms; Nex: 4; Flip angle: 90; TSE factor: 25; FOV: 260/183/80; Matrix: 512x262; 32 contiguous slices; Slice thickness: 2.5 mm), 2D Fast Field Echo T2-weighted (TR/TE: 646/23 ms; Nex: 2; Flip angle: 18; FOV: 250/188/73; Matrix: 304x171; 20 slices; Slice thickness: 3 mm), all in coronal planes.

Diffusion MR imaging

Diffusion-weighted imaging was performed in coronal planes using a single shot Spin-Echo-EPI (Echo Planar Imaging) sequence with b values of 0 and 1000 ms (TR/TE: 3002/89 ms; Nex: 1; Flip angle: 90; EPI factor: 47; FOV: 230/230/92; Matrix: 112x89; 23 contiguous slices; Slice thickness: 4 mm). ADC (Apparent Diffusion Coefficient) values were obtained in 7 regions of interest (ROI), using Osirix Software (anterior and posterior part of the preoptic and tuberal region of the right and left hypothalamus, right and left thalamus, and lateral ventricle for the ADC of the cerebro-spinal fluid or CSF). The surface of the ROI was 3 mm^2, for a slice thickness of 4mm. The voxel size was thus of 12 mm^3. One single rater placed the diffusion ROIs to minimize potential observer bias in ROIs placement that may occur randomly. The rater was familiar with the anatomical delineation of the hypothalamic area on ADC maps after training with a senior neuroradiologist. The ADC values were obtained bilaterally both in the hypothalamus and the thalamus, were averaged and normalized to the ADC values from the CSF (ADC Thalamus/ADC CSF; ADC Hypothalamus/ADC CSF).

Inclusion of little amounts of CSF could have occurred during the placement of the ROI within the hypothalamus. However, the putative inclusion of CSF in the Hypothalamic ROIs was random and thus very unlikely to influence the results. Importantly, CSF ADC values did not show any statistical difference neither between sampling (paired t-test, $p > 0.05$ between women at two stages of their artificial ovarian cycle) nor between subjects (ANOVA, $p > 0.05$ between male and female subjects).

Proton MR spectroscopy

Spectroscopy was performed using the same magnet as the one used for MRI in the same setting. Following morphological examination, spectra were acquired using a single voxel PRESS (Point resolved spectroscopy) technique (TR/TE: 2000/31 ms; voxel size 10x10x10 mm^3; Nex= 144; half echo acquisition). The water signal was suppressed using a CHESS (chemical shift selective) technique. One voxel was located within the hypothalamus (Figure 1A) and another one within the thalamus (Figure 1B). Both voxels were arbitrarily positioned on the left side for right-handed volunteers and on the right side for left-handed volunteers. Data were post-processed using the advanced signal-processing tool for Medical Magnetic Resonance Imaging and Spectroscopy, Java Magnetic Resonance User Interface (jMRUI v2.2; 2005; www.mrui.uab.es/mrui/mrui_Overview.shtml). The post processing steps in the time domain were truncation (the removal of 2 points at the beginning of the echo), frequency shift (the correction from the residual water peak frequency (4.77 ppm)), zero filling of factor 2 (1024 to 2048) points apodization with Lorentzian filter (factor 5), and spectrum hardphase with water reference and baseline correction. A second frequency shift from the N-acetyl aspartate (NAA) top of the peak (set to 2.02 ppm) was applied in the frequency domain. Spectrum limits were set from 0 to 4 ppm. For quantitation, prior knowledge parameters included chemical shift range for Creatine (Cr; 2.99-3.06 ppm) and Choline (Cho; 3.19-3.26 ppm). The area under the peak was estimated for NAA, Cr and Cho, using AMARES (advanced method for accurate, robust, and efficient spectral fitting) (Vanhamme et al., 1997). The NAA/Cr, Cho/Cr, Cho/NAA, NAA/Cho+Cr ratios were calculated separately for

the hypothalamus and for the thalamus. The signal to noise ratio (SNR) was recorded for each spectrum as well.

Experimental design

The influence of low dose combined oral contraceptives on hypothalamic structure and metabolic changes was investigated in ten female volunteers with a mean age of 21.3 ± 1.0 (s.d.) years and a body mass index (BMI) of 20.9 ± 1.5, by measuring the apparent diffusion coefficient (ADC) of water molecules and the content of some key metabolites using diffusion magnetic resonance imaging and spectroscopy, respectively. Female volunteers were subjected to MRI at two stages of their artificial menstrual cycle: 13 days (d13) after initiating oral contraception and at the end of the pill-free interval (d-2). A group of 10 male volunteers with a mean age of 21.7 ± 1.7 years and a BMI of 22,7 ± 2.7 were also included in the study.

Statistical analysis

Data were subjected to statistical tests using the SPSS software (11.5) for Windows XP. A paired t-test was used to compare data acquired at the two different stages of the artificial menstrual cycle in women. Statistically significant differences between male and female volunteers were analyzed using ANOVA followed by Student-Newman-Keuls multiple range test. The level of statistical significance was set at less than 0.05.

Results

We first investigated the influence of low dose combined oral contraceptives on hypothalamic structure by measuring in ten female volunteers the apparent diffusion coefficient (ADC) of water molecules using diffusion magnetic resonance imaging (dMRI) (Le Bihan, 2003). A group of ten male volunteers were also included in the study. As detailed in the materials and methods section, ADC values were obtained bilaterally in two regions of interest (ROI) within the hypothalamus (Fig. 1a,b) and one within the thalamus (Fig. 1c) and

averaged. ADC values obtained in the cerebrospinal fluid (CSF) of the lateral ventricle (Fig. 1a,b) were used for normalization (Table 1). Figure 1d shows that while no difference was noted between sexes (ANOVA, $p > 0.05$, $n = 10$ per group), the mean ADC was significantly increased during the pill free period (d-2) when compared to oral contraceptive use (d13) (paired t-test, $p = 0.015$, $n = 10$) in the hypothalamus of female subjects. Interestingly, as no changes were monitored within the thalamus (Fig. 1e; paired t-test, $p > 0.05$ d-2 *vs.* d13, $n = 10$), removal of the tonic steroidogenic break on the hypothalamo-pituitary-gonadal axis during the pill free period appears to selectively increase diffusivity within the hypothalamus.

We then proceeded with the identification of putative metabolic changes evoked by the artificial menstrual cycle in the human brain. Proton MR spectroscopy, which measures freely mobile molecules (Ross and Bluml, 2001), was performed on the aforementioned healthy group of young adults. An experimental voxel was placed within the hypothalamus (Fig. 2a), while a control voxel of the same volume included the ipsilateral thalamus (Fig. 2a). The data from female subjects showed a significant decrease in the ratios of concentration of choline (Cho, 3.22 ppm, Fig. 2b) to N-acetyl-aspartate (NAA, 2.02 ppm, Fig. 2b) during the pill free period (d-2) when compared to both the period of oral contraceptive use (d13) (paired-t-test, $p < 0.05$, $n = 10$) and males (ANOVA, $p = 0.01$, $n = 10$) in the hypothalamus (Fig. 2d), but not in the thalamus (Fig. 2e). Because both NAA/creatine (Cr, 3.01 ppm) and choline/creatine ratios remain unchanged (Table 2), our results suggest that hypothalamic choline levels are diminished during the pill free interval.

Discussion

The main finding of this study is that withdrawal of the negative feedback exerted by gonadal steroids on the hypothalamo-pituitary-gonadal axis selectively promotes both an increase in diffusion and metabolic changes in the hypothalamus. Our results stand in contrast with those of a recent positron emission tomography (PET) study that failed to monitor changes in [^{18}F]fluorodeoxyglucose uptake as a measure of metabolic activity during gonadal steroid-promoted LH secretion in postmenoposal women within the hypothalamus (Ottowitz et al.,

2008). However, the key concept behind PET is the indirect (and still poorly understood) link between local blood flow and neuronal activation through metabolism (Barros et al., 2005; Le Bihan, 2007; Nehlig and Coles, 2007). In contrast with this approach, the diffusion MRI and MR spectroscopy techniques used in the present study have the potential to reveal changes in the intrinsic water physical properties and cerebral metabolite release, respectively, during brain activation, which could be more intimately linked to the neuron-glia activation mechanisms and retain a better spatial and temporal resolution (Le Bihan, 2007). Further, it should be noted that Ottowitz et al. (2008) analyzed hypothalamic response to sex steroids in women that underwent reproductive senescence, thus possible postmenoposal alteration in hypothalamic ability to sense changes in gonadal steroid levels cannot be excluded.

The longitudinal study of healthy young female volunteers allowed us to determine that diffusion of water molecules in the hypothalamus changes during the artificial menstrual cycle and that diffusivity is maximal at the pill-free period when normal early follicular phase LH pulsatile release is found (Hemrika et al., 1993). The basic mechanism underlying the increase in diffusion remains unclear. Different hypotheses have been suggested, such as a possible increase in extracellular and intracellular water mobility, a shifting of water from the intracellular to the extracellular space, a decrease in the restriction of intracellular diffusion due to changes in membrane permeability, or a decrease in the tortuosity of the extracellular space due to cell retraction (Norris et al., 1994). Interestingly, studies conducted in brain slices have shown that changes in the astrocytic coverage of neurons modify extracellular space geometry and diffusion parameters, such as tortuosity in discrete hypothalamic nuclei during physiological activation (Piet et al., 2004). Rodent studies have also shown that the amplification in the pulsatile release of GnRH, which drives LH secretion, might be a consequence of the structural remodeling of hypothalamic GnRH neural networks during the onset of the preovulatory surge (Herbison, 2006). Increases in water ADC during the pill-free period could thus reflect transient microstructural changes involving neurons and/or glial cells in the hypothalamus, which translate into gonadal axis activation.

As revealed by *in vivo* measurements of brain metabolites with quantitative proton MR spectroscopy, artificial menstrual cycle also promoted metabolic changes within the hypothalamus. As the ratios of the concentration of Choline to NAA was significantly decreased in the pill-free phase compared to that measured during the use of oral contraception, while both choline/creatine and NAA/Cr ratios remain unchanged, our results suggest that hypothalamic choline levels are diminished during the pill free interval. Alterations in choline levels have been thought to result from changes in cytosolic choline compounds produced by disturbances in the formation and degradation of cell membranes. Since choline is concentrated in astrocytes (Urenjak et al., 1993; Manganas et al., 2007), one could speculate about a reduction in the cell size of astroglial cells ensheathing GnRH neurons (Witkin et al., 1991; King and Letourneau, 1994; Prevot et al., 1999; Yamamura et al., 2004) and/or other hypothalamic neurons (Garcia-Segura et al., 1994; Gao Q and Priest CA, 2007), as seen in non-human primates, rodents and birds at stages when circulating levels of gonadotropins are rising. That glial cells may play a role in the control of GnRH secretion in humans is suggested by a recent neuroanatomical study demonstrating that GnRH neurons morphologically interact with astroglial cells in the hypothalamus of humans (Baroncini et al., 2007), as it does in other species (Ojeda et al., 2000).

Our data provide *in vivo* imaging evidence for sex steroid hormone-driven plasticity in the female human hypothalamus. These MRI-based physiological studies, which yield insights into the neural processes that respond to sex steroid hormones to regulate reproduction in humans, may pave the way for the development of new diagnostic and treatment strategies in the central loss of reproductive competence in human syndromes, such as hypothalamic amenorrhea.

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Legends

Figure 1. Greater diffusivity in the hypothalamus in the absence of oral contraceptives. **a–c.** Localization of the region of interest (green lines, 3 mm^2) in which the apparent diffusion coefficient (ADC) was measured. Left and right panels show coronal planes of diffusion MRI and T1 weighted MRI, respectively, containing the anterior (**a**) and posterior (**b**) parts of the hypothalamus and the thalamus (**c**). cc, corpus callosum; Cd, caudate nucleus; fx, fornix; mpo, medial preoptic nucleus; opt, optic tract; bcc, body of the corpus callosum; LV, lateral ventricle; ic, internal capsule; arc, arcuate nucleus; cp, cerebral peduncle; Th, thalamus. **d.** and **e.** Box plot representation of the ratio of hypothalamic ADC/ cerebrospinal fluid (CSF) ADC values and of thalamic ADC/CSF ADC values, respectively, measured in women (n = 10) at two stages of their artificial menstrual cycle (d-2: two days before use of oral contraception in the pill free interval; d13, 13 days after initiating oral contraception) and in men (n = 10). Values depicted are the median for each group with the upper and lower limits of the box representing the 75^{th} and 25^{th} percentile, respectively. The error bars represent the 95^{th} and the 5^{th} percentiles. The dot (d) and the stars (e) point to outliers, i.e., observations that are numerically distant from the rest of the data. *P* values were obtained from paired t-tests comparing d-2 and d13.

Figure 2. The absence of oral contraceptives triggers a decrease in the ratio of concentration of choline (Chol) to that of N-acetyl aspartate (NAA) in the female hypothalamus during the artificial menstrual cycle. **a.** Placement of the voxel (red lines, 1 cm^3) used for MR spectroscopic analysis within the hypothalamus (upper panels) and the thalamus (lower panels) as shown in coronal (left panels), axial (middle panels) and sagittal (right panels) planes. cc, corpus callosum; Cd, caudate nucleus; fx, fornix; opt, optic tract; LV, lateral ventricle; cp, cerebral peduncle; Th, thalamus. **b.** Representative proton MR spectroscopy spectrum of hypothalamus along with molecular assignment of resonances (NAA : N acetyl aspartate at 2.02 ppm ; Cr : creatine at 3.01 ppm; Cho : choline at 3.22 ppm). **c.** Fits of the individual metabolites within the proton MR spectroscopy spectrum shown in **b**. Areas under

the curve correspond to the quantities of metabolites. **d.** and **e.** Box plots summarizing Cho/NAA ratios in women at d-2 and d13 of their oral contraception cycle (n = 10), and in men (n = 10) in the hypothalamus and thalamus, respectively. *P* values were obtained from Student paired t-tests and ANOVA for d-2 *vs.* d13 women and men *vs.* d-2 women, respectively.

Table 1. Ratio of the apparent diffusion coefficient (ADC) of the Hypothalamus (HT) or Thalamus (TH) to that of the CSF in women both at d-2 (Group 1) and at d13 (Group 2) of their artificial menstrual cycle, and men (Group 3). R, right; L, Left.

	Group 1 (d-2)		Group 2 (d13)		Group 3 (men)		Statistical analysis		
	Mean	SEM	Mean	SEM	Mean	SEM	*p* 1 *vs.* 2	*p* 1 *vs.* 3	*p* 2 *vs.* 3
ADC HT R / CSF	0.273	0.013	0.257	0.012	0.256	0.017	**0.008**	0.458	0.966
ADC HT L / CSF	0.257	0.013	0.247	0.010	0.251	0.011	0.058	0.730	0.785
ADC HT / CSF	0.265	0.013	0.252	0.011	0.254	0.014	**0.015**	0.561	0.929
ADC TH / CSF	0.251	0.014	0.251	0.013	0.238	0.012	0.988	0.494	0.468

Table 2. Ratios of metabolite concentrations in the hypothalamus and thalamus.

		Group 1 (d-2)		Group 2 (d13)		Group 3 (men)		Statistical analysis		
		Mean	SEM	Mean	SEM	Mean	SEM	*p* 1 *vs.* 2	*p* 1 *vs.* 3	*p* 2 *vs.* 3
Hypothalamus	NAA / Cr	2.375	0.321	2.503	0.458	1.655	0.391	0.672	0.172	0.176
	Cho / Cr	0.736	0.138	1.693	0.410	0.878	0.106	0.079	0.423	0.070
	Cho / NAA	0.330	0.070	0.714	0.128	0.737	0.123	**0.046**	**0.010**	0.896
	NAA / (Cho + Cr)	1.398	0.175	0.975	0.147	0.869	0.203	0.057	0.064	0.677
Thalamus	NAA / Cr	3.366	0.390	4.254	1.101	2.237	0.416	0.516	0.063	0.104
	Cho / Cr	1.410	0.441	1.271	0.324	1.566	0.589	0.621	0.835	0.666
	Cho / NAA	0.458	0.148	0.380	0.077	0.719	0.254	0.560	0.386	0.218
	NAA / (Cho + Cr)	1.714	0.277	1.875	0.408	1.019	0.145	0.754	**0.039**	0.064

5. Figure 1

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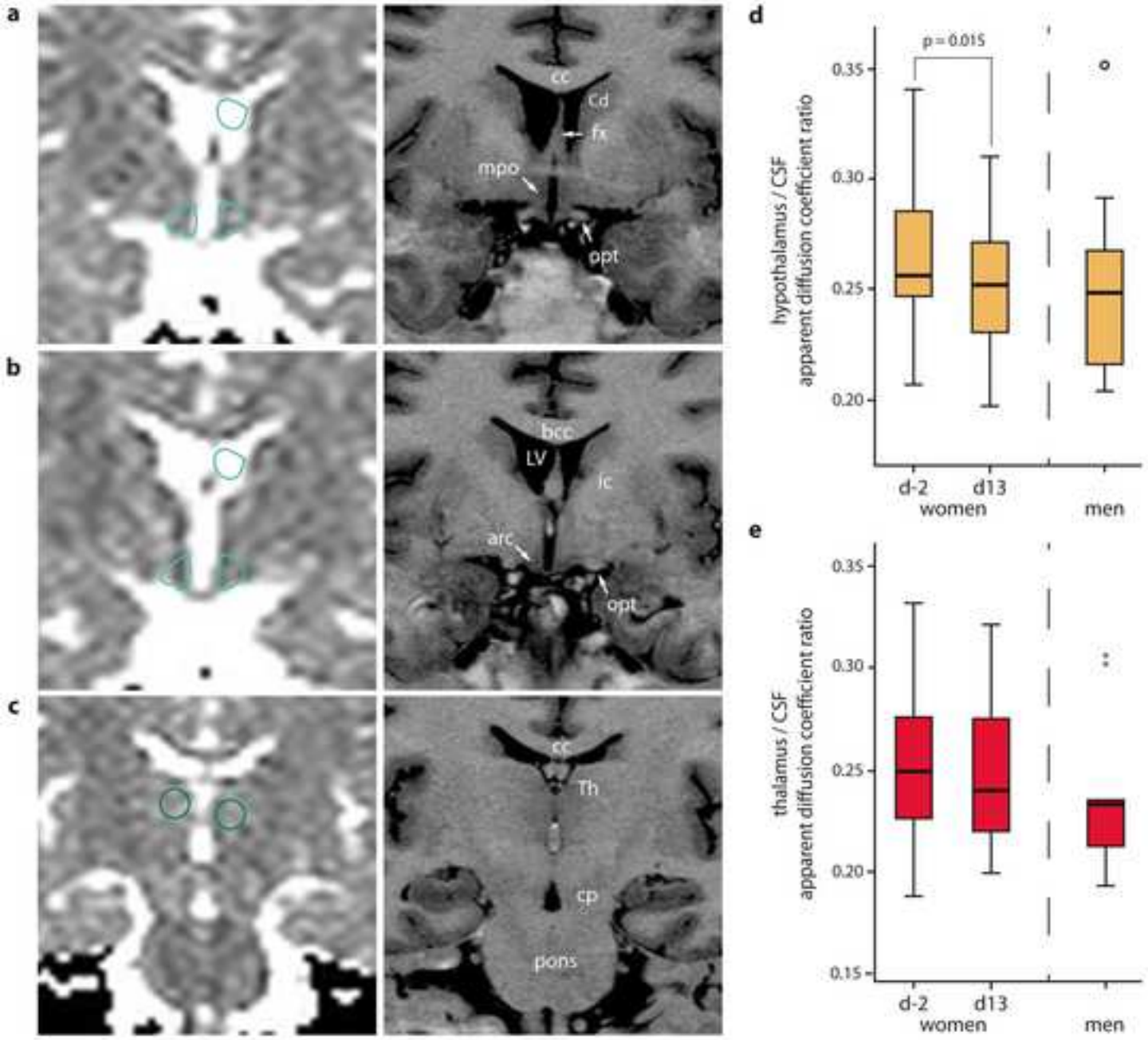

Figure 1

5. Figure 2

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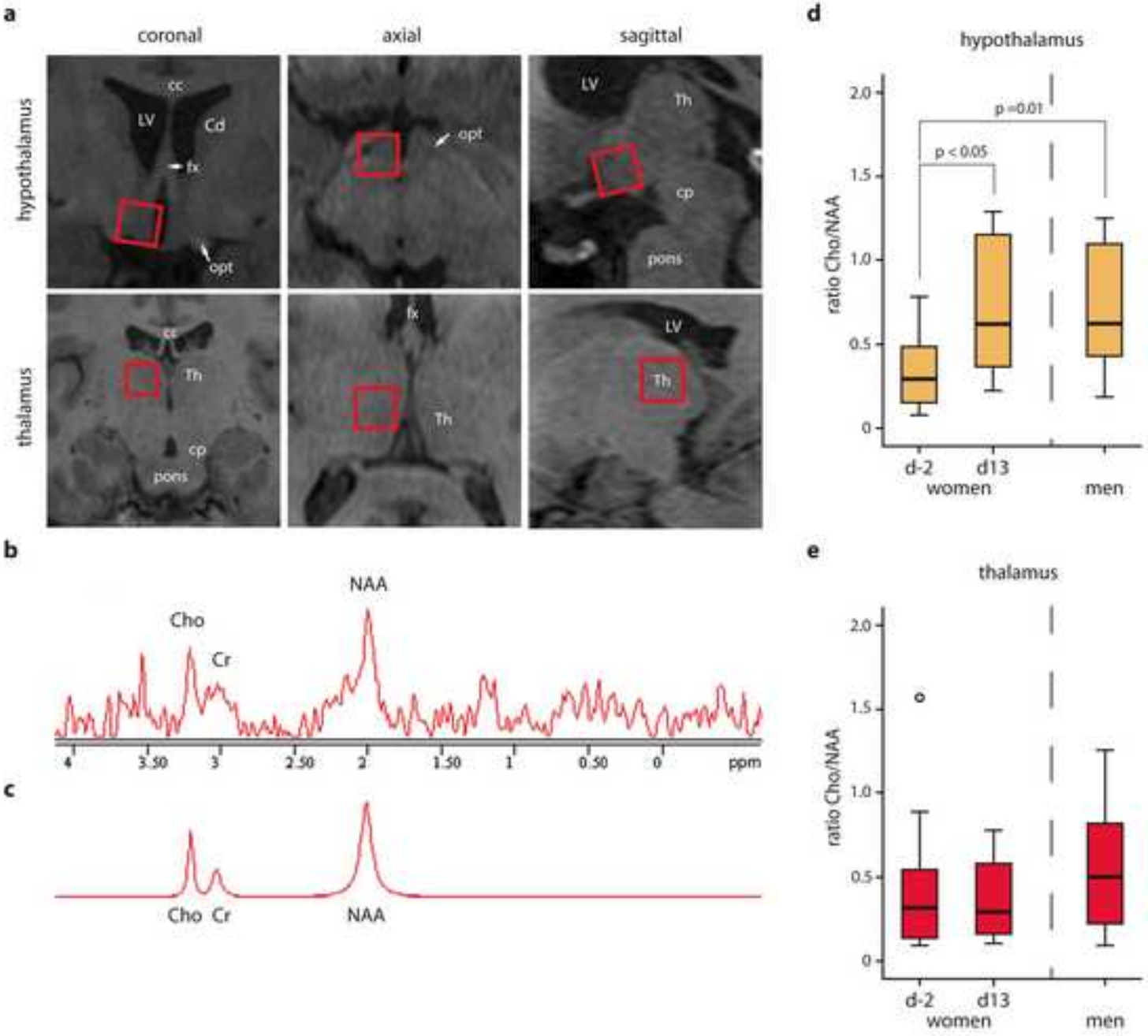

Figure 2