NeuroImage 83 (2013) 593–598

Contents lists available at ScienceDirect

NeuroImage

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

Hippocampal volumes differ across the mouse estrous cycle, can change within 24 hours, and associate with cognitive strategies

Lily R. Qiu [a,1], Jürgen Germann [a,1], Shoshana Spring [a], Christina Alm [b], Dulcie A. Vousden [a], Mark R. Palmert [b,c,d], Jason P. Lerch [a,e,*]

[a] Mouse Imaging Centre, Program in Neuroscience and Mental Health, The Hospital for Sick Children, Toronto, Ontario, Canada
[b] Division of Endocrinology, The Hospital for Sick Children, Toronto, Ontario, Canada
[c] Department of Pediatrics, University of Toronto, Toronto, Ontario, Canada
[d] Department of Physiology, University of Toronto, Toronto, Ontario, Canada
[e] Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada

ARTICLE INFO

Article history:
Accepted 25 June 2013
Available online 3 July 2013

ABSTRACT

Recent human and rodent brain imaging studies have shown that the shape of the brain can be changed by experience. These mesoscopic alterations in neuroanatomy are hypothesized to be driven by changes at the level of neuronal processes. To examine whether the shape of the brain changes rapidly, we used MRI to examine changes in the volume of the hippocampus across the 4–6 day estrous cycle in the female mouse. It is well known that changing steroid levels across the cycle influence dendritic spine maturation and alter synapse density in the hippocampus; our results show that the estrous cycle is associated with approximately 2–3% changes in hippocampal volume as seen by high-resolution ex-vivo MRI. Changes in hippocampal volume are, moreover, associated with a switch between hippocampal and striatal based navigation strategies in solving the dual choice T-maze in the same mice. A second experiment, using in-vivo MRI, suggests that these changes in hippocampal volume can occur over a 24 hour period. In summary, we show that the brain is highly plastic at a mesoscopic level of resolution detectable by MRI, that volumetric increases and decreases in hippocampal volume follow previously established patterns of changes in neuropil, and that these changes in volume predict changes in cognition.

Introduction

There are intriguing indications that the volume and shape of the human brain can change over short periods of time. Direct evidence includes increases in local gray matter volume in the parietal and occipital cortices when learning to juggle (Draganski et al., 2004; Driemeyer et al., 2008; Scholz et al., 2009) or acquiring similar dexterity skills (Taubert et al., 2012). There is, however, significant controversy over the reproducibility of plasticity related brain changes detected by MRI (Thomas and Baker, 2012); and the underlying mechanism is uncertain (Zatorre et al., 2012). Understanding how the brain changes at a mesoscopic resolution — i.e. at the level of brain regions and nuclei — is a key challenge for the brain imaging community, as it will both (i) answer fundamental questions of how the brain is shaped and how malleable that shape is, and (ii) provide further understanding of abnormal brain plasticity found in multiple neurologic and psychiatric diseases.

Part of the answer will come from work in animal models, where high-field MRI can provide the link between the rich literature of cellular brain plasticity in model systems and the emerging field of human systems-level brain plasticity. We have shown, for example, that 5 days of training mice on the Morris water maze changes the volume of the hippocampus and striatum by 2–4% as detected by high-field MRI and correlates with an immunohistochemical stain for neuronal process remodelling (Lerch et al., 2011b). Similarly, maze training in the rat has shown diffusion related brain changes associated with astrocyte immunoreactivity and synapse staining (Blumenfeld-Katzir et al., 2011; Sagi et al., 2012).

The dominant paradigm in studying mesoscopic brain plasticity has been to use an external stimulus, such as training on mazes or dexterity tasks, and use imaging to examine the outcomes of these stimuli on the brain. Brain plasticity, however, can also occur in response to endogenous signals; to, for example, cycling hormone levels.

Ovarian steroids have been well established to affect the brain (McEwen and Alves, 1999), in particular the hippocampus with its high density of estrogen receptors (Woolley, 1998). Estradiol induces maturation in dendritic spines in the rodent hippocampus, and has been associated with increases in synaptic markers in rodents and primates (Spencer et al., 2008a; Woolley and McEwen, 1992). Over

* Corresponding author at: The Hospital for Sick Children, 25 Orde St., Toronto, ON, M5T 3H7, Canada.
E-mail address: jason.lerch@sickkids.ca (J.P. Lerch).
[1] These authors contributed equally.

1053-8119/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neuroimage.2013.06.074

the approximately 4–6 days of the rodent estrous cycle, synapse markers peak at proestrus when estradiol levels are high, decline rapidly over a 24 hour period as the cycle enters estrus where estradiol and progesterone levels are low, and then rise gradually over the next several days in metestrus and diestrus (Spencer et al., 2008a; Woolley, 1998) (illustrated in Fig. 1a). There is also an intriguing association between the estrous cycle and cognition: during proestrus, when estradiol is high, rats are much more likely to choose a hippocampal dependent place strategy to solve a maze than during estrus, when steroid levels are low and a non-hippocampal response strategy is more common (Korol and Kolo, 2002; Korol et al., 2004).

In this study we thus set out to address the following hypothesis: the neuronal alterations known to occur with changing steroid levels across the 4–6 day estrous cycle will be associated with changes in hippocampal volume as seen by MRI between estrous cycle stages. Furthermore, we expect that these volume changes will be associated with a concomitant alteration in the cognitive strategy used to solve a maze, with larger hippocampi during proestrus associated with use of a place strategy.

Materials and methods

We conducted two experiments. In the first, mice were assessed for their estrous cycle stage, trained on a T-maze to determine which cognitive strategy they used to solve the maze, and then sacrificed and their brains imaged with high-resolution ex-vivo MRI.

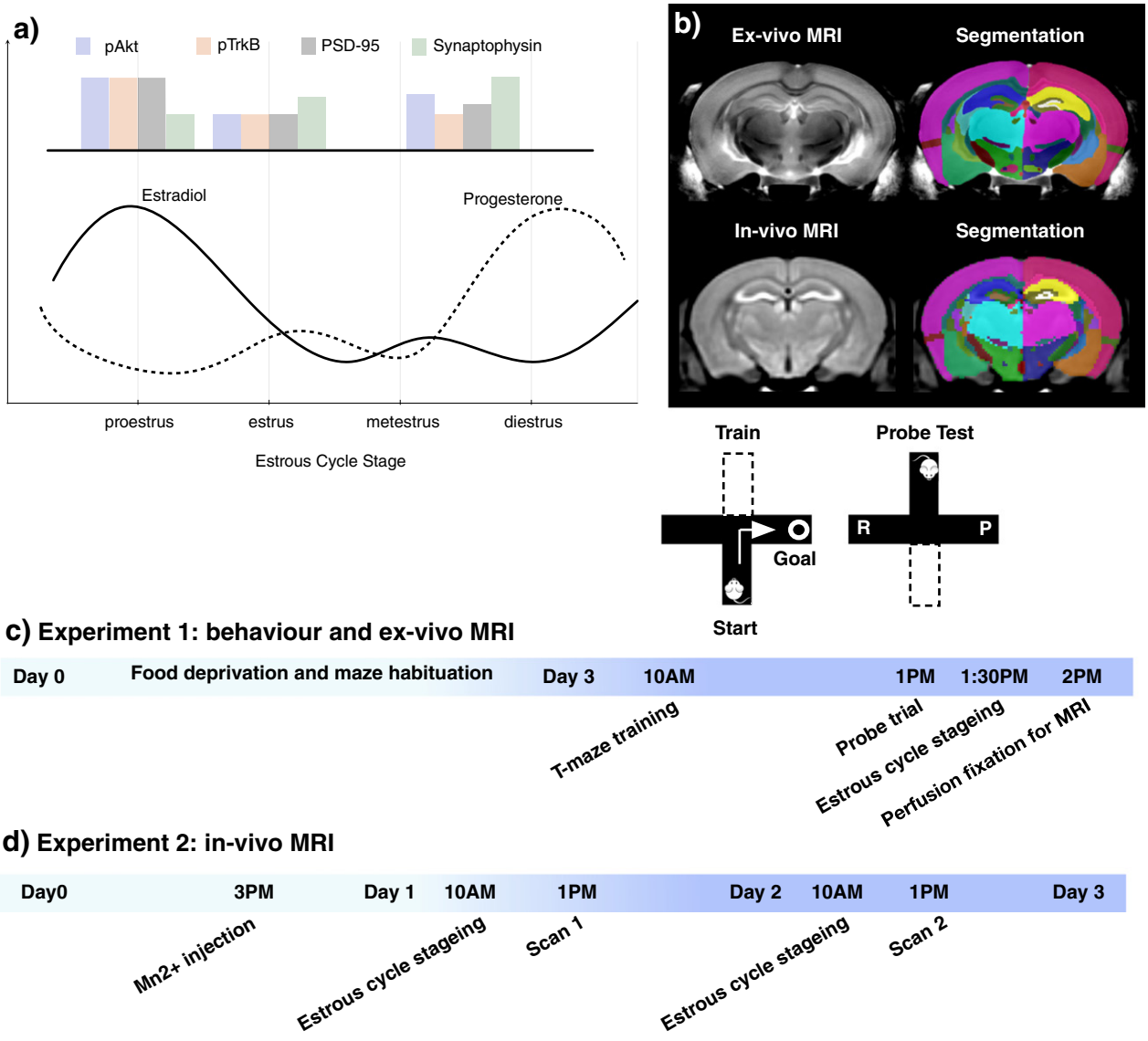

Fig. 1. An outline of hypotheses and experimental design. As has been repeatedly shown, the estrous cycle is associated with an increase in estradiol toward proestrus followed by a sharp decline in estrus. These changes in steroids affect the morphology of dendritic spines and numbers of synapses as indicated by multiple synaptic markers, as shown in (a). During proestrus, when estradiol peaks, post-synaptic markers (PSD-95) and phosphorylated Akt (pAkt), which leads to new spine formation, are highest, as is the Brain Derived Neurotrophic Factor (BDNF) receptor TrkB (pTrkB). These values all rapidly decrease in estrus, and begin to rise again through metestrus and diestrus. Conversely, synpatopysin, a pre-synaptic marker, is relatively low in proestrus and increases toward diestrus. These data are synthesized and adapted from McLean et al. (2012) and Spencer et al. (2008a). We imaged mice across the estrous cycle and automatically measured volumes of the hippocampus. Sample ex-vivo and in-vivo images and the corresponding atlas segmentations are shown in (b) and the experimental design in (c–d). For the ex-vivo experiment, mice were habituated to the T-maze and food deprived to 85% of their starting body weight over three days. On the fourth day, mice were trained on the T-maze and their spontaneous navigation strategy assessed on a probe trial. If the mouse made the same direction turn (i.e. left or right) when starting in the opposite arm for the probe trial it was considered to be using a response strategy; if the mouse used the spatial cues in the room and made the correct turn for the food reward, it was considered to be using a place strategy. After the probe trial the mice were perfusion fixed for ex-vivo MRI. For the in-vivo experiment, no behavioural testing was performed. Mice instead were injected with Mn^{2+} the day before the first in-vivo imaging session, and imaged a second time 24 hours later. This 3 day procedure was performed starting on post-natal day 76 (with imaging on days 77 and 78).

This experiment allowed us to relate hippocampal volume to estrous cycle stage and cognition. In the second experiment, mice were imaged at different stages of the estrous cycle using in-vivo Manganese Enhanced MRI (MEMRI) to measure hippocampal volume change over a 24 hour period. Methods and experimental design are illustrated in Fig. 1. All experiments were approved by the Animal Ethics Committee of the Toronto Centre for Phenogenomics.

Mice

For the ex-vivo experiment, 39 female C57BL/6 mice, aged 74–95 days, were used during varying stages of their estrous cycle (proestrus $n = 8$, estrus $n = 9$, metestrus $n = 13$, diestrus $n = 9$). For the in-vivo experiment, 20 female C57BL/6 mice were imaged twice each on post-natal days 77 and 78. On day 77 the estrous cycle was proestrus $n = 5$, estrus $n = 2$, metestrus $n = 7$, diestrus $n = 6$. Mice were obtained through in-house breeding at the Toronto Centre for Phenogenomics or ordered from Jackson Laboratories.

T-maze habituation

For the first ex-vivo experiment mice were food deprived for 3 days prior to and including the day of testing. They were weighed each day to ensure that no mice fell below 85% of their initial weight. For each of the 3 days prior to testing, each mouse was given 10 minutes to openly explore the T-maze with food at the arms to allow habituation of both the maze and food reward. The 20 mice used for the second in-vivo experiment did not undergo maze habituation, training or testing.

Maze training

Noldus' Ethovision XT was used to program the protocol for the T-maze. Mice were trained to enter one of the arms to obtain a food reward until 9/10 consecutive trials were completed successfully. Immediately after training, a probe trial was administered in which the start box was rotated 180 degrees from the original location. Four spatial cues (triangle, circle, etc.) were placed around the T-maze apparatus at fixed locations that did not change from initial training to probe, or between trials with different mice. Response learning was displayed if the mouse incorrectly made the same direction turn (i.e. right or left) during the probe trial as during initial training. Place learning was demonstrated if the mouse made the correct turn to obtain the food reward, i.e. the opposite turn from that during initial training. From this behavior we inferred that the mouse used the spatial cues around the T-maze to navigate to the baited arm. A second observer, blinded to the estrous stage of the mice, viewed video recordings of 10 separate probe trials to ensure inter-rater reliability for cognitive strategy assessment.

Estrous cycle stage identification

To determine the estrous cycle stage of each mouse vaginal secretions were obtained from each mouse. For the ex-vivo study, mice were staged on each of the 3 days of habituation to increase the accuracy of the estrous cycle stage identification on the day of training. On the day of T-maze training, mice were staged after the training was completed (approximately 2 PM in the afternoon). Mice were restrained by the tail, upside down, to allow placement of a plastic pipette tip inside the vagina. The pipette was filled with 30 μL of saline, and was flushed in and out of the vagina three to five times. The final flush was placed on a glass slide and viewed under a light microscope under 10× magnification. Each stage was characterized by the dominance of a cell type: proestrus was predominantly nucleated epithelial cells; estrus contained anucleated cornified cells; metestrus contained leukocytes, cornified and nucleated epithelial cells; diestrus consisted predominantly of leukocytes (McLean et al., 2012). Staging was identical for the in-vivo study, except that the vaginal secretions were collected at 10 AM, prior to administration of anaesthesia and imaging in the afternoon.

Preparation for ex-vivo brain imaging

After estrous cycle staging, mice were anaesthetized by an intraperitoneal injection of a ketamine (100 mg/kg) and xylazine (20 mg/kg) solution. Mice were then transcardially perfused through the left ventricle with 30 mL of phosphate-buffered saline (PBS), 1 μL/mL Heparin and 2 mM ProHance at a flow rate of 1.0 mL/minute. This was followed by infusion of 30 mL 4% paraformaldehyde (PFA) and 2 mM ProHance at 1.0 mL/minute for fixation. After perfusion, the head along with skin, lower jaw and ears were removed in order to isolate the brain, still in the skull. Following dissection, skulls were placed in individual vials of 4% PFA and 2 mM ProHance solution overnight at 2 °C. After at least 24 hours, skulls were transferred to a solution of 4% PFA, 0.02% sodium azide and 2 mM ProHance.

Ex-vivo MR imaging

A multi-channel 7.0 Tesla, 40 cm diameter bore magnet MRI scanner (Varian Inc. Palo Alto, CA) was used to acquire images of mouse brains. Brains were intact in their skulls and placed in Fluorinert, and 16 samples were scanned at one time in a 16-coil solenoid array. Parameters used were: a T2-weighted 3D fast spin-echo sequence, with TR = 2000 ms, echo train length = 6, TEeff = 42 ms, field-of-view = 25 × 28 × 14 mm and matrix size = 450 × 504 × 250 (Cahill et al., 2012; Lerch et al., 2011a).

In-vivo MR imaging

We used manganese enhanced MRI (MEMRI) to measure neuroanatomical changes within mice occurring across 2 consecutive days. Mice were injected intraperitoneally with 30 mM manganese chloride ($MnCl_2$) solution in fractionated doses (0.4 mmol/kg): one half of the total dose at 18 hours and the second half at 17 hours before the first scan. Mice were scanned 7 at a time in separate transmit/receive coils two times, 24 hours apart. Prior to each scan, mice were anaesthetized with 4% isoflurane and placed within the magnet bore for imaging. Mice were maintained at a body temperature of 35 °C on 1% isoflurane. Mn-enhanced images were acquired using a spoiled gradient echo sequence with TR = 0.1 s, TE = 3.7 ms, flip angle = 55°, field-of-view = 35 × 21 × 21 mm, matrix size = 280 × 168 × 168, and two averages for a total of 1 hour and 34 minutes scan time. Post-scanning, mice were transferred to a heated cage to allow for recovery from the anaesthetic.

Image registration and measures of hippocampal volume

In-vivo and ex-vivo images were analyzed separately. In each case a fully automated image registration based pipeline was used to align all scans into spatial correspondence (Lerch et al., 2011a). A digital atlas was then used to compute volumes for 62 separate structures in the brain (Dorr et al., 2008). The volume of the hippocampus, defined to include CA1, CA2, and CA3, was used as the main outcome measure. For the ex-vivo data, hippocampal volume was normalized and measured as a percentage of whole brain volume; for the in-vivo data, the difference in volume (as a percentage of brain volume) between adjacent scan days (i.e. day 78 minus day 77) was computed.

Statistical analysis

For the ex-vivo data, estrous stage was treated as an ordered factor based on reported estradiol and progesterone levels (estrus < metestrus < diestrus < proestrus) (McLean et al., 2012; Spencer et al., 2008a,b), and a linear model with $F_{(3,\ 35)}$ degrees of freedom used to assess the relation between hippocampal volume and cycle stage. For the in-vivo data, the transition between stages (i.e. metestrus to diestrus) for each mouse on the 2 scan days was determined. Mice grouped into four possible categories: (1) starting in an hypothesized high hippocampal volume stage of the estrous cycle (proestrus and diestrus) and remaining in a presumed high hippocampal volume stage, (2) starting in a high hippocampal volume stage and going to low hippocampal volume stage (estrus and metestrus), (3) starting in a low volume stage and remaining in a low volume stage, and (4) starting in a presumed low volume stage and transitioning to a high volume stage. A Welch two sample *t*-test with 10.2 degrees of freedom assessed whether volume changed when going from a hypothesized high to low volume stage versus moving from an hypothesized low to high volume stage. For the behavioral data, differences in proportions of mice using a place or response strategy in each stage of the estrous cycle was assessed using a χ^2 test with 3 degrees of freedom.

Results

Behavior

There was no difference by estrous stage in the number of trials needed to reach criteria ($p = 0.78$, Fig. 2a). The probe trial revealed a significant difference in the proportion of mice using a place strategy ($p = 0.03$, Fig. 2b). There was perfect consistency between raters on assessing navigation strategy on the probe trial (10/10).

Hippocampal volume

Hippocampal volume, as assessed by high-resolution ex-vivo MRI, was significantly related to estrous cycle stage ($p = 0.004$, Fig. 2c), with the maximum difference between adjacent stages reaching 2.8%. Hippocampal volume furthermore predicted navigation strategy on the T-maze ($p = 0.03$), with larger volume predicting the use of a place strategy and smaller volume the use of a response strategy.

Twenty different female mice were then imaged twice each in-vivo and differences in hippocampal volume across the 24 hour time period between adjacent scans computed. The difference in hippocampal volume across 24 hours increased when going from a stage with low baseline hippocampal volume (estrus or metestrus)

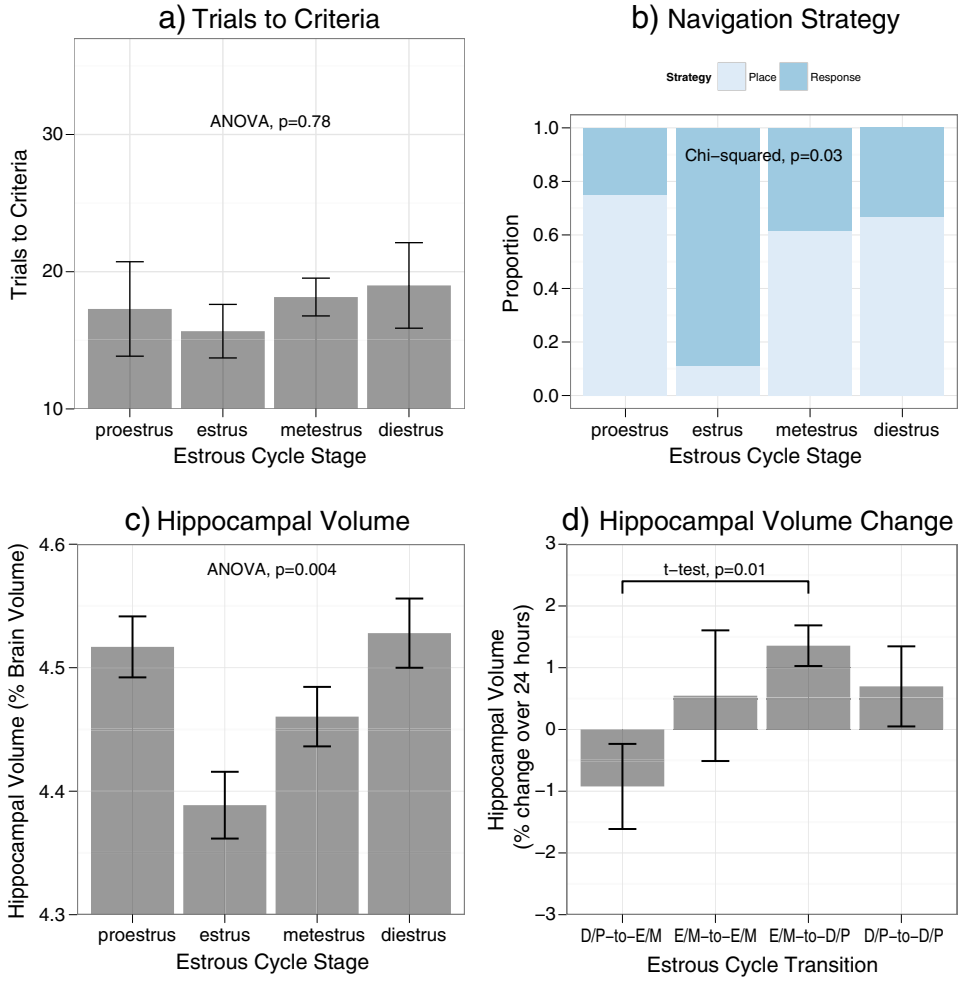

Fig. 2. Results. (a) There was no difference across estrous cycles stages in the mice's ability to solve the T-maze ($p = 0.78$). Nevertheless, the strategy used to solve the maze differed across the cycle ($p = 0.03$), with mice in estrus least likely to use a place strategy (b). The estrous cycle stage each mouse was in further related directly to ex-vivo measured hippocampal volume ($p = 0.004$), with volume being lowest in estrus and increasing until proestrus (c). A separate cohort of mice was then imaged in-vivo and differences in hippocampal volume determined across a 24 hour time period. As seen in (d) volume increased when mice were in estrus/metestrus transitioning to diestrus/proestrus, and decreased if transitioning from diestrus/proestrus to estrus/metestrus ($p = 0.01$). All error bars are SEM.

to high baseline hippocampal volume (proestrus or diestrus) and decreased when transitioning from a baseline of high hippocampal volume to low hippocampal volume. Total hippocampal volume change in transitioning between stages, as measured by in-vivo MRI, was 2.2% and was significant ($p = 0.01$, Fig. 2d).

Discussion

Here we show that hippocampal volume in female mice changes during the estrous cycle, mirroring known alterations in steroids across the cycle and relating directly to behavior. This constitutes direct evidence that the mesoscopic anatomy of the brain is capable of changing in ways detectable by MRI over short periods of time.

We used two separate experiments to relate hippocampal volume to the estrous cycle. In the first experiment, a cohort of 39 young adult female mice were tested on a T-maze to assess their propensity to use a hippocampus-dependent place strategy to solve the maze. After maze training, which takes approximately 30–45 minutes per mouse, the stage of the estrous cycle was assessed, following which the animal was sacrificed and the brains prepared for high resolution ex-vivo MRI. This experiment allowed us to determine that, given a population of young female mice, hippocampal volume differs dependent on the estrous cycle. These differences follow known steroid levels, with hippocampal volume lowest in the estrus phase, and as estradiol and progesterone levels increase toward their peak in proestrus and diestrus respectively so does hippocampal volume. These alterations in hippocampal volume tie into behavior; we showed that mice in estrus are most likely to use a response strategy to solve the T-maze (as previously reported in rats (Korol and Kolo, 2002; Korol et al., 2004)), with likelihood for using a place strategy increasing with hippocampal volume and known presence of ovarian steroids during proestrus and diestrus.

The first experiment suggested that hippocampal volume changes between the four stages of the estrous cycle. In the second experiment we determined that changes in hippocampal volume across the estrous cycle can be detected in-vivo and occur within a 24 hour period. We divided our mice into four groups based on expected hippocampal volume on day 1 and day 2. We found that hippocampal volume did indeed decrease between the two scans if the mouse started in a stage with presumed high volume (proestrus and diestrus) and transitioned to a stage with presumed low volume (estrus and metestrus) whereas volume increased if going from presumed low to high volume stages.

The effects of estrogen and to a lesser extent progesterone on the hippocampus have been well studied at a cellular level, giving us important clues about the origin of the volume changes we have shown here. The dominant, often reported morphological change is an approximately 30% increase in dendritic spines in rats in late proestrus (when estradiol is high) compared to late estrus (when estradiol is low) (Woolley, 1998; Woolley and McEwen, 1992, 1994). Alterations in spine numbers in mice across the estrous cycle are not known, though multiple synaptic markers (PSD-95, pAkt, Synpatophysin) alter during the cycle (Spencer et al., 2008a) and an increase in estradiol results in greater numbers of multi-synaptic boutons and in larger "mushroom" shaped spines (Li et al., 2004; Spencer et al., 2008b). The alterations in synaptic markers are concentrated in area CA1 of the hippocampus in rats, but appear to affect the entire hippocampus more evenly in mice (Spencer et al., 2008b). There is also some suggestion that estradiol increases astrocyte volume (Spencer et al., 2008b), though this is not as well replicated (Woolley, 1998). In summary, the large alterations in spines and synapses that have been well replicated in the rodent are highly likely to underlie the volumetric changes we report using MRI.

The purported link between spines/synapses and mesoscopic volume is well in line with the two previous studies that have linked rodent MRI brain plasticity with cellular markers (Blumenfeld-Katzir et al., 2011; Lerch et al., 2011a,b; Sagi et al., 2012). When we trained mice on a water maze we were able to detect hippocampal volume increases of ~3–4% which correlated with GAP-43, a marker of neuronal process remodelling (Lerch et al., 2011b). Similarly, when Blumenfeld-Katzir and colleagues trained rats on a water maze, alterations in water diffusion properties were linked with changes in astrocyte as well as synapse staining intensity in the hippocampal formation (Blumenfeld-Katzir et al., 2011; Sagi et al., 2012). The 2–3% increase in hippocampal volume reported in the current study, where endogenous estrous cycling with its well established effect on synapses likely drove the volumetric changes, further cements the link between alterations at the level of neuronal and glial processes and MR detectable volume.

There are a few caveats to our study in so far as our experimental setup could subtly alter natural cycling and/or steroid levels in mice. The food deprivation required for the behavioural testing might be disruptive as could, more severely, the anaesthesia involved in the in-vivo MRI protocol. Indeed, in the in-vivo experiment, not all mice transitioned to the next stage in the cycle, though this could also be due to each cycle stage not necessarily being 24 hours long. We were therefore underpowered to look at all possible stage transitions and thus grouped our mice into two cycle stages (proestrus/diestrus with presumed high steroid levels and hippocampal volume, and estrus/metestrus with presumed low steroid levels and hippocampal volume) and transitions between them. The experimental protocols, however, require food deprivation and anaesthesia and thus will unavoidably be disruptive to some extent. A further caveat is that we did not measure steroid levels in individual animals. The relation between cycle stage and steroid levels has been well established, yet future work could break down variation within stages by correlating brain morphometry against each mouse's estrogen and progesterone levels.

In summary, the experiment described above adds to our understanding of mesoscopic brain plasticity as follows:

- Mesoscopic brain plasticity can be rapid – we found the hippocampus changes in volume within 24 hours.
- The fact that mesoscopic brain plasticity is rapid suggests that it may be driven by membrane-initiated signalling, since new gene products from nucleus-initiated signalling take over 24 hours (Spencer-Segal et al., 2012), and is related to dendritic spine maturation and synapse numbers (Li et al., 2004; Spencer et al., 2008a).
- Brain shape is rapidly modified by endogenous neuromodulators (i.e. ovarian steroids), as suggested by human imaging studies (Protopopescu et al., 2008), as well as by the previously established learning/training paradigms (Blumenfeld-Katzir et al., 2011; Lerch et al., 2011b).
- The changes in hippocampal volume tightly relate to changes in cognition. Previous data from our group showed that directed spatial learning in a water maze grows the hippocampus if the mice are forced to rely on distal spatial cues to find the escape platform (Lerch et al., 2011b); in the current study, on the other hand, endogenous signalling changes the volume of the hippocampus which in turn biases learning strategies when both a place (hippocampal) or response (striatal) strategy are available.

Our work has important implications for neurocognitive investigations using structural MRI to measure changes in the brain. Most importantly, the fact that the brain can change so rapidly suggests that examining changes in the brain within hours or days, as opposed to the usual time period of weeks or months, could be promising. For human imaging studies, where conventional longitudinal paradigms designed to detect changes in the brain image every few weeks or months, designing experiments examining short term changes in the brain could be significantly more powerful, since isolating changes to just the experimental manipulations as opposed to all the other activities of daily living the subjects carry out will be

significantly easier. As the methods used by the brain imaging community become more sensitive, whether due to improved acquisition methods at higher fields or better data analysis algorithms, we believe that the brain will be found to be constantly but subtly changing in response to endogenous (i.e. steroids) as well as external factors.

Conflicts of interest

There are no conflicts of interest to declare.

References

Blumenfeld-Katzir, T., Pasternak, O., Dagan, M., Assaf, Y., 2011. Diffusion MRI of structural brain plasticity induced by a learning and memory task. PLoS One 6, e20678.

Cahill, L.S., Laliberté, C.L., Ellegood, J., Spring, S., Gleave, J.A., van Eede, M.C., Lerch, J.P., Henkelman, R.M., 2012. Preparation of fixed mouse brains for MRI. Neuroimage 60, 933–939.

Dorr, A.E., Lerch, J.P., Spring, S., Kabani, N., Henkelman, R.M., 2008. High resolution three-dimensional brain atlas using an average magnetic resonance image of 40 adult C57Bl/6J mice. Neuroimage 42, 60–69.

Draganski, B., Gaser, C., Busch, V., Schuierer, G., Bogdahn, U., May, A., 2004. Neuroplasticity: changes in grey matter induced by training. Nature 427, 311–312.

Driemeyer, J., Boyke, J., Gaser, C., Büchel, C., May, A., 2008. Changes in gray matter induced by learning—revisited. PLoS One 3, e2669.

Korol, D.L., Kolo, L.L., 2002. Estrogen-induced changes in place and response learning in young adult female rats. Behav. Neurosci. 116, 411–420.

Korol, D.L., Malin, E.L., Borden, K.A., Busby, R.A., Couper-Leo, J., 2004. Shifts in preferred learning strategy across the estrous cycle in female rats. Horm. Behav. 45, 330–338.

Lerch, J.P., Sled, J.G., Henkelman, R.M., 2011a. MRI phenotyping of genetically altered mice. Methods Mol. Biol. 711, 349–361.

Lerch, J.P., Yiu, A.P., Martinez-Canabal, A., Pekar, T., Bohbot, V.D., Frankland, P.W., Henkelman, R.M., Josselyn, S.A., Sled, J.G., 2011b. Maze training in mice induces MRI-detectable brain shape changes specific to the type of learning. Neuroimage 54, 2086–2095.

Li, C., Brake, W.G., Romeo, R.D., Dunlop, J.C., Gordon, M., Buzescu, R., Magarinos, A.M., Allen, P.B., Greengard, P., Luine, V., McEwen, B.S., 2004. Estrogen alters hippocampal dendritic spine shape and enhances synaptic protein immunoreactivity and spatial memory in female mice. Proc. Natl. Acad. Sci. U. S. A. 101, 2185–2190.

McEwen, B.S., Alves, S.E., 1999. Estrogen actions in the central nervous system. Endocr. Rev. 20, 279–307.

McLean, A.C., Valenzuela, N., Fai, S., Bennett, S.A.L., 2012. Performing vaginal lavage, crystal violet staining, and vaginal cytological evaluation for mouse estrous cycle staging identification. J. Vis. Exp. 67, e4389. http://dx.doi.org/10.3791/4389.

Protopopescu, X., Butler, T., Pan, H., Root, J., Altemus, M., Polanecsky, M., Mcewen, B., Silbersweig, D., Stern, E., 2008. Hippocampal structural changes across the menstrual cycle. Hippocampus 18, 985–988.

Sagi, Y., Tavor, I., Hofstetter, S., Tzur-Moryosef, S., Blumenfeld-Katzir, T., Assaf, Y., 2012. Learning in the fast lane: new insights into neuroplasticity. Neuron 73, 1195–1203.

Scholz, J., Klein, M.C., Behrens, T.E.J., Johansen-Berg, H., 2009. Training induces changes in white-matter architecture. Nat. Neurosci. 12, 1370–1371.

Spencer, J.L., Waters, E.M., Milner, T.A., McEwen, B.S., 2008a. Estrous cycle regulates activation of hippocampal Akt, LIM kinase, and neurotrophin receptors in C57BL/6 mice. Neuroscience 155, 1106–1119.

Spencer, J.L., Waters, E.M., Romeo, R.D., Wood, G.E., Milner, T.A., McEwen, B.S., 2008b. Uncovering the mechanisms of estrogen effects on hippocampal function. Front. Neuroendocrinol. 29, 219–237.

Spencer-Segal, J.L., Tsuda, M.C., Mattei, L., Waters, E.M., Romeo, R.D., Milner, T.A., McEwen, B.S., Ogawa, S., 2012. Estradiol acts via estrogen receptors alpha and beta on pathways important for synaptic plasticity in the mouse hippocampal formation. Neuroscience 202, 131–146.

Taubert, M., Villringer, A., Ragert, P., 2012. Learning-related gray and white matter changes in humans: an update. Neuroscientist 18, 320–325.

Thomas, C., Baker, C.I., 2012. Remodeling human cortex through training: comment on May. Trends Cogn. Sci. (Regul. Ed.) 16, 96–97 (author reply 97–8).

Woolley, C.S., 1998. Estrogen-mediated structural and functional synaptic plasticity in the female rat hippocampus. Horm. Behav. 34, 140–148.

Woolley, C.S., McEwen, B.S., 1992. Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. J. Neurosci. 12, 2549–2554.

Woolley, C.S., McEwen, B.S., 1994. Estradiol regulates hippocampal dendritic spine density via an *N*-methyl-D-aspartate receptor-dependent mechanism. J. Neurosci. 14, 7680–7687.

Zatorre, R.J., Fields, R.D., Johansen-Berg, H., 2012. Plasticity in gray and white: neuroimaging changes in brain structure during learning. Nat. Neurosci. 15, 528–536.