Low CA1 Spine Synapse Density is Further Reduced by Castration in Male Non-human Primates

Csaba Leranth[1,2], Janine Prange-Kiel[1,4], Karyn M. Frick[3] and Tamas L. Horvath[1,2]

[1]Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT 06520, USA, [2]Department of Neurobiology, Yale University School of Medicine, New Haven, CT 06520, USA, [3]Department of Psychology, Yale University, New Haven, CT 06520, USA and [4]Institute of Anatomy, University of Hamburg, D-20246 Hamburg, Germany

The hippocampus plays a major role in learning and memory and its morphology and function are readily affected by gonadal hormones in female non-human primates. We sought to determine whether the gonads also affect pyramidal cell spine synapse density in the CA1 hippocampal area of male primates. Unbiased electron microscopic stereological calculations were performed to determine the volumetric density of pyramidal cell spine synapses and semiquantitative analyses on the surface density of glial fibrillary acidic protein-containing glia processes and the diameter of pyramidal cell apical dendrites in the CA1 area of intact and orchidectomized (1 month) St Kitts vervet monkeys (*Chlorocebus aethiops sabaeus*). The volumetric density (number of spine synapse/μm^3) of spine synapses was significantly lower (40%) in the gonadectomized animals than in control monkeys; conversely, the density of glia processes was significantly higher (15%) and the diameter of dendritic shafts located in this area was also larger (30%) in the orchidectomized animals than in the controls. Strikingly, when compared to female values, intact male primates had lower spine synapse densities than either intact or ovariectomized females. Since the primate hippocampus is very similar to that of a human's, the present observations suggest that physiological levels of circulating androgen hormones are necessary to support normal spine synapse density in the CA1 stratum radiatum of human male hippocampus.

Keywords: electron microscopic unbiased stereological calculation, glial fibrillary acidic protein, orchidectomy, pyramidal cell dendrite, sexual differences, vervet monkey

Introduction

Recent data have demonstrated that experimental manipulations and physiological changes in the levels of circulating female gonadal hormones greatly influence the density of pyramidal cell spine synapses in the CA1 hippocampal subfield of both female rats (Gould *et al.*, 1990; Woolley *et al.*, 1990, 1997; Woolley and McEwen, 1992, 1993; Leranth and Shanabrough, 2001; Leranth *et al.*, 2000) and monkeys (Leranth *et al.*, 2002). Interestingly, the integrity of the male hippocampus also requires normal levels of circulating male hormones. For example, it has been shown that testosterone (T), but not estrogen (E) has a synaptoplastic effect on the hippocampus of gonadectomized male rats (Leranth *et al.*, 2003). In addition, it is well established that a large population of neurons in the monkey hippocampus, similar to the rat hippocampus (Simerly *et al.*, 1990; Brown *et al.*, 1995) contains androgen receptors (Pomerantz and Sholl, 1987; Choate *et al.*, 1998; Abdelgadir *et al.*, 1999; Roselli *et al.*, 2001). In the rat hippocampus, androgen receptors appear to be primarily located in pyramidal neurons (Clancy *et al.*, 1992; Kerr *et al.*, 1995). Because both the rat and monkey hippocampus contain low levels of aromatase (MacLusky *et al.*, 1994; Roselli *et al.*, 2001), effects of T could be mediated by either actions on androgen receptors or conversion to E. Although major differences have been shown in the morphology and functional connections of the hippocampus between monkeys and rats, great similarities exist in this respect between monkeys and humans (e.g. Rosene and Van Hoesen, 1987), so the findings presented here may have significant implications for humans. Therefore, this study aimed to define whether hormonal manipulations influence synaptic plasticity in the hippocampus of male subhuman primates. Furthermore, we wished to address the question of whether the same hormonal manipulation influences the density of astroglia processes in the same brain region, as is seen in rats (Lam and Leranth, 2003).

Materials and Methods

Young adult (5–6 years; 5.6–6.3 kg) male (n = 6) St Kitts vervet monkeys (*Chlorocebus aethiops sabaeus*) were used. The animals were housed in individual cages (water and regular monkey chow were provided in excess of nutritional needs) at the St Kitts Biomedical Research Foundation, St Kitts (West Indies). The facility is in full compliance with all applicable US regulations, and the treatment and care of these monkeys were in compliance with the Guide for the Care and Use of Laboratory Animals (1996) US Public Health Service, Washington, DC, National Academy Press. Experimental protocols were approved by the Institutional Animal Care and Use Committee of Yale University Medical School and Axion Research Foundation. All efforts were made to minimize discomfort and pain and the number of animals used. Half of the monkeys were anesthetized (ketamine sedation, 10 mg/ kg, i.m., followed by 20 mg/kg pentobarbital, i.v.) and orchidectomized (GDX), under sterile conditions. One month later, before killing the animals (ketamine sedation 10 mg/kg i.m., followed by an overdose, 100 mg/kg of pentobarbital, i.v.), blood was collected and centrifuged; each sample was divided into three aliquots, placed into liquid nitrogen and shipped to Yale. To minimize measuring errors, serum T level was measured in each aliquot using radioimmunoassay; the sensitivity of the radioimmunoassay used is <0.2 ng/ml.

Thereafter, the chest was opened, the descending aorta clamped, the right atrium opened and after rinsing the vascular system via transcardial perfusion using a motorized peristaltic pump (Masterflex L/S; Cole Palmer, San Francisco, CA) with 1000 ml 0.9% heparinized (1 unit/ml) saline, animals were further perfused for 10 min with 1500 ml of fixative containing 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.35; PB). The temperature of both solutions was 20°C. Brains were postfixed at 4 °C for 2 h in the same fixative and stored in PB at 4 °C. The hypothalami and the nigrostriatal systems of these animals were used in other studies. The hippocampi were dissected out and divided into three equal pieces along its longitudinal axis. Thereafter, small 2–3 mm long blocks were cut (perpendicular to the longitudinal axis) out from the middle portion of these tissue samples and 50 and 200 μm thick sections were cut perpendicular to the longitudinal axis of the hippocampal formation on the vibratome for light microscopic immunostaining and electron microscopic statistical analyses, respectively.

Immunostaining for Glial Fibrillary Acidic Protein (GFAP)

In order to eliminate unbound aldehydes, the 50 μm vibratome sections were pretreated with 1% sodium borohydride in PBS for 30 min (Sigma, St Louis, MO) and washed 3 × 10 min in PB. Thereafter, sections were incubated in the primary antibody – rabbit anti-GFAP (DAKO, Carpinteria, CA), 1:1000 in PB containing 2% normal goat serum (ICN Biomedicals, Aurora, OH) and 0.5% Triton X-100 (Acros Organics, Fairlawn, NJ) – overnight at room temperature. Sections were washed 3 × 10 min in PB and further incubated in the secondary antibody – biotinylated goat anti-rabbit IgG (Vector Laboratories Burlingame, CA), 1:500 in PB for 2 h at room temperature – followed by another rinse in PB (3 × 10 min) and an incubation in the ABC Elite reagents (1:500; Vector Laboratories Burlingame, CA). After washing in PB (3 × 10min), the tissue bound peroxidase was visualized using nickel–diaminobenzidine (Ni–DAB: 15 mg DAB, 12 mg ammonium chloride, 0.12 mg glucose oxidase, 600 μl 0.05 M nickel ammonium sulphate and 600 μl 10% β-D-glucose in 40 ml PB) for 8 min. Sections were mounted on gelatin-coated slides, dehydrated and mounted in Permount. To ensure that sections taken from the gonadectomized and control animals were treated under identical conditions, they were incubated together throughout the procedures.

Light Microscopic Analysis

The stratum radiatum of the hippocampal CA1 subfield was examined. Data analysis was performed as described recently (Lam and Leranth, 2003). Briefly, from each GFAP-immunostained section (25/animal), non-overlapping photographs (five per section) of the region were taken using 100× oil immersion and 20× objective lenses for process and cell counting, respectively, under an Olympus BX60 microscope equipped with a Zeiss AxioCam digital camera. The location of the first photograph was randomly selected, then the other four were taken from areas located on the left- or the right-hand side of the first photographed field, depending on the location of the first photograph. For analysis, images were further processed using Adobe Photoshop 6.0 and printed out at a final magnification of 160× (process counting) or 32× (cell counting) on a Brother HL-1450 laser printer. For process counting, a 100 × 80 μm large grid, composed of square grid lines 10 μm apart, was laid on the print. Immunoreactive processes in focus crossing the grid lines were counted to determine the relative density of processes as described previously (Horvath *et al.*, 1999; Lam and Leranth, 2003). Twenty pictures from each animal were analyzed and the mean for each animal was calculated from these data. For cell counting, only GFAP positive cells in focus with clearly distinguishable somata were counted in a total area of 150 × 500 μm on each print; in this case 10 pictures were analyzed and used to determine the mean per animal. In order to avoid a biased analysis, sections were encoded prior to the analysis.

Electron Microscopic Analysis

For electron microscopic examination, the 200 μm vibratome sections (taken for the aforementioned three major areas along the longitudinal axis of the hippocampus) were post-osmicated (1% OsO_4 in PB, for 30 min), dehydrated in increasing concentrations of ethanol (the 70% bath contained 1% uranyl acetate, for 30 min) and embedded in Araldite. Consecutive serial sections prepared from the middle third of the CA1 stratum radiatum (see Fig. 1 in Leranth *et al.*, 2002) of each vibratome section were collected on Formvar-coated single slot grids. Sections were analyzed and photographed in a Tecnai 12 electron microscope equipped with an AMT Advantage 4.00 HR/HR-B CCD camera system (Advanced Microscopy Techniques, Danvers, MA).

Because synaptic density could be affected by changes in the volume of the tissue, a correction factor was first calculated by following the steps of a widely accepted protocol (Rusakov *et al.*, 1997). This method is based on the assumption that the hormonal manipulations did not influence the total number of CA1 pyramidal cells. As performed previously (Leranth *et al.*, 2000, 2002), six or seven disector pairs (pairs of adjacent 2 μm semithin sections; 'reference' and 'look-up' sections) which contained the CA1 pyramidal cell layer were cut on the ultratome from Araldite blocks (three blocks, taken from three different areas along the longitudinal axis of the hippocampal formation, see above), of all hippocampi and placed on slides. Sections were stained with toluidine blue. The cell layer was located in the field of view of the light microscope (Olympus BX60 microscope furnished with a Zeiss AxioCam digital camera connected to a computer) and observed also on the screen before being captured as a TIFF file. The slide was then moved to allow the adjacent area to be captured. This process was repeated for the entire CA1 pyramidal cell layer of both the reference and look-up sections. In each of the captured images, a pair of parallel lines (250 μm apart) perpendicular to the layer was placed to form a sampling window. The simultaneous observation of captured images on the screen allowed the alignment of sampling windows in the reference and look-up sections. Thereafter, the disector technique was applied to count the cell nuclei in both sections. Only those cell nuclei were counted that were present in one, but not on the other section and vice versa (Braendgaar and Gundersen, 1986). The left hand 'window line' served as 'exclusion line' (see Figs 1A,B in Rusakov *et al.*, 1997). The cell density value (D) was calculated using the formula $D = N/sT$, where N is the mean disector score across all the sampling windows, T is the thickness of the sections (2 μm) and s stands for the length of the window. Based on these values, a dimensionless volume correction factor kv was introduced: $kv = D/D1$, where $D1$ is the mean density across the different animals.

Photographs (at 9900× magnification) were made from identical fields of pairs of consecutive serial ultrathin sections ('reference' and 'look-up' sections) from areas located between the upper and middle third of the CA1 stratum radiatum (300–500 μm from the pyramidal cell layer) with the observer blinded to the experimental treatment. As in the light microscopic analysis, the location of the first photograph was randomly selected. Thereafter, non-overlapping photographs were taken from the left- and right-hand sides of the first photographed area. Potentially interfering structures, such as blood vessels, large dendrites or glial cells, were intentionally avoided. In order to obtain a comparable measure of synaptic numbers, unbiased for possible changes in synaptic size, the disector technique (Braendgaar and Gundersen, 1986) was used. Thus, the density of spine synapses [which were identified by the lack of mitochondria, but the presence of spine apparatus and dark postsynaptic bar adjacent to the synaptic contact in the head of the spines (Gray type 2 synapses) and vesicle accumulation at the synaptic contact in the presynaptic bouton] of pyramidal cell apical dendrites was calculated with the help of a reference grid superimposed on the EM photographs. The disector volume (volume of reference) was the unit area of the reference grid multiplied by the distance between the upper faces of the reference and look-up sections. Section thickness (average 0.075 μm) was determined by using the electron scattering technique (Small, 1968). Only those spine synapses were counted that were present on the reference section, but absent in the corresponding look-up section and vice versa (Fig. 1). The measured synaptic density values were divided by the volume correction factor kv. This correction provided a synaptic density estimate normalized with respect to the density of pyramidal cells and also accounted for possible changes in hippocampal volume.

At least five neuropil fields were photographed on each electron microscopic grid. With at least eight grids from each vibratome section (containing a minimum of two pairs of consecutive, serial ultrathin sections), each animal provided 5 × 8 × 2 = 80 neuropil fields. Each pair (photos of reference and look-up sections) of photographs represented an 8.9 × 8.9 × 0.075 μm = 5.94 μm^3 volume. As mentioned, from each monkey 80 photographs were analyzed that represented a 475 μm^3 volume.

In addition to the spine synapse density calculation, a semiquantitative analysis was performed on the diameter of dendrites located in this area. On all prints used in the spine density calculation, the diameter of dendritic profiles (30 dendrites in randomly selected photos taken from each animal) were measured and compared.

Statistical Analyses

For all dependent variables (glia process density, dendritic diameter and spine synapse density), the means determined for each animal were used for the statistical analysis. The non-central F-statistic was used to test the difference in the ratios of means between groups and group means were compared using pairwise t-tests (two-tailed proba-

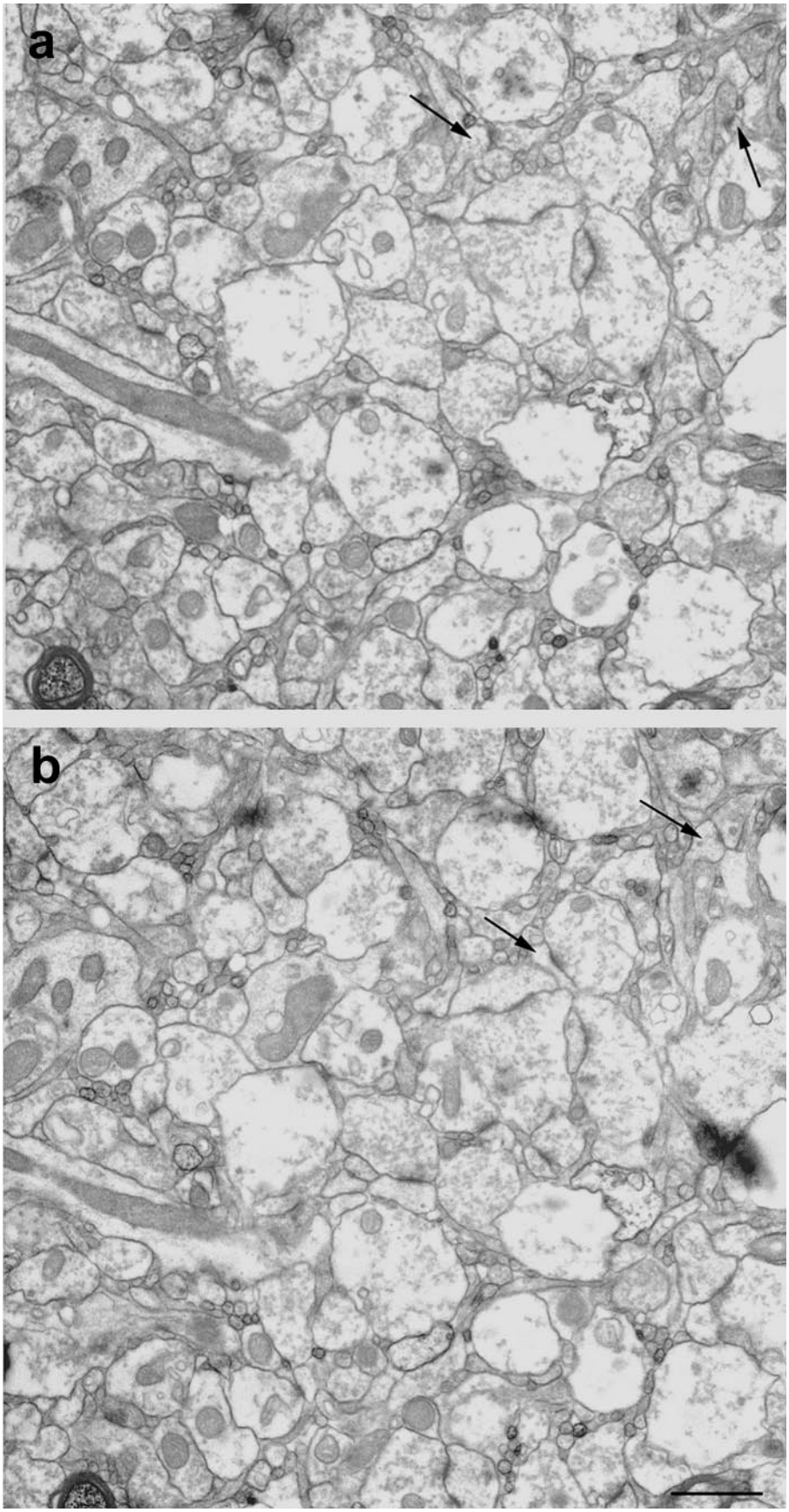

Figure 1. Electron micrographs demonstrate two consecutive serial sections: a reference (*a*) and look-up section (*b*). Only those spine synapses (arrows) were counted that were present in just one section. Scale bar = 1 μm.

bilities). A level of confidence of $P < 0.05$ in two-tailed tests was adopted. *P*- and *t*-values as well as the degrees of freedom (d.f.) are given in the figure legends. In the text, the mean ± SEM values are given.

Results

Serum T Levels

There was a major difference between the levels of circulating T in the GDX and control monkeys. In GDX monkeys, the mean T level was <0.2 ng/ml (the minimum sensitivity of radioimmunoassay is <0.2 ng/ml). In control monkeys, the average serum T level was 1.134 ± 0.016 ng/ml (Table 1), which is the same as we measured previously in the serum of same-age St Kitts male monkeys and very similar to the lower end of T levels measured in human men.

Astroglia Process Density

Astroglia processes could easily be detected due to the specific GFAP staining. Light microscopic observations already suggested a difference in the density of astroglial processes between the control and GDX monkeys. In the CA1 of GDX animals, the fiber density was increased compared to the control (Fig. 2). The semiquantitative analysis confirmed this optical impression (Fig. 3). In control animals, the mean density of glia processes was 108.8 ± 2.952/8000 μm^2, whereas in the castrated animals it was 124.8 ± 3.926/8000 μm^2 (Table 1). This relative increase (15%) in the density of glia processes was statistically significant ($P < 0.031$). In contrast, there was no significant difference in the density of GFAP-containing cell bodies in the CA1 regions of castrated and control animals (data not shown).

Spine Synapse Density

No significant differences were observed in the density of pyramidal cell nuclei between the control (840 ± 73.901/mm^2) and GDX animals (832 ± 64.663/mm^2; Table 1). Furthermore, in the electron microscope, no obvious qualitative differences could be observed by comparing the ultrastructure of CA1 stratum radiatum of control and GDX monkeys. However, surveying all of the electron micrographs, it appeared that the density of small glia processes is higher in the material depicted from GDX monkeys than in the control animals. Because of technical difficulties and the extremely time-consuming nature of a meaningful electron microscopic statis-

Table 1

Data demonstrating: CA1 area of pyramidal-cell, spine-synapse, glia-cell and glia-process density; diameter of pyramidal cell apical dendrites, in the upper third of the stratum radiatum; and levels of serum testosterone in individual control and castrated male monkeys

Parameters	Control	Castrated
Mean pyramidal cell density (mm^2)	840	832
	968	720
	712	944
Mean spine synapse density (/475 μm^3)	260	168
	288	157
	276	164
Mean corrected spine synapse density (/μm^3)	0.547	0.353
	0.6059	0.330
	0.5820	0.3450
Mean glia cell density (/75 000 μm^2)	22.1	24.1
	25.8	18.9
	16.7	23.7
Mean glia process density (/8000 μm^2)	103.10	117.35
	110.40	130.58
	112.95	126.70
Mean diameter of dendritic shafts (μm)	0.99	1.318
	1.035	1.39
	1.05	1.284
Level of serum testosterone (ng/ml)	1.167	<0.2
	1.115	<0.2
	1.121	<0.2

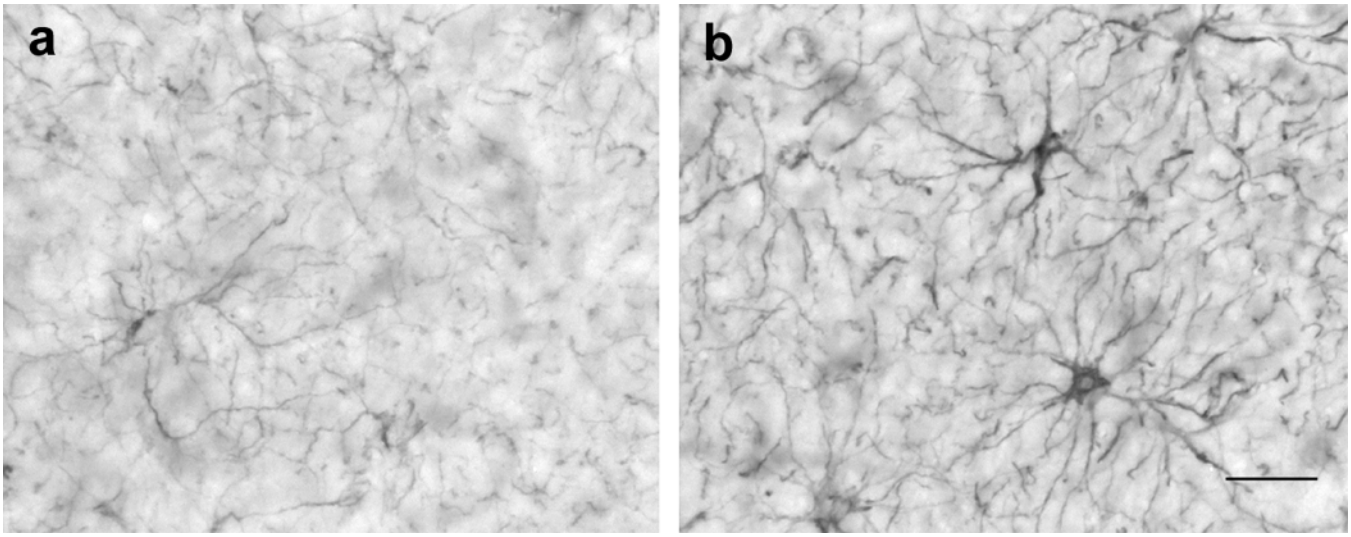

Figure 2. Light micrographs show glial fibrillary acidic protein (GFAP)-immunoreactive glia cells and processes in vibratome sections taken from the stratum radiatum of the CA1 hippocampal subfield of a control (*a*) and a 1 month orchidectomized monkey (*b*). Note the much higher density of immunoreactive glia profiles in the hippocampus of the gonadectomized animal (*b*). Scale bar = 50 μm.

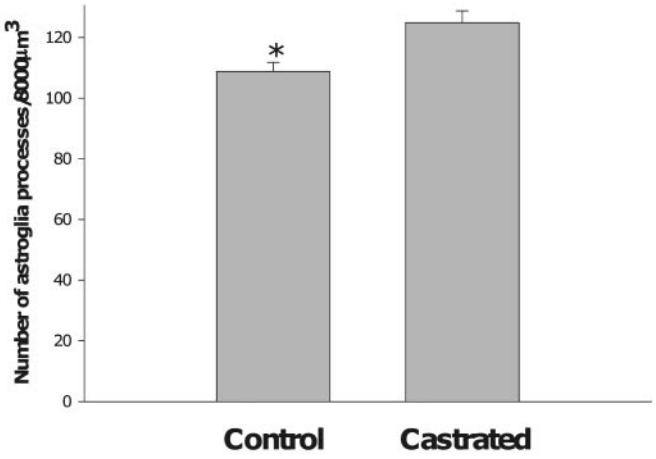

Figure 3. Bar graphs demonstrate the result of a semiquantitative calculation on the density of glia processes in the stratum radiatum of the CA1 hippocampal subfield. The surface density of glia processes is significantly higher (15%) in the orchidectomized (1 month) than control monkeys (mean ± SEM, $*P < 0.031$, $t = -3.270$, d.f. = 4).

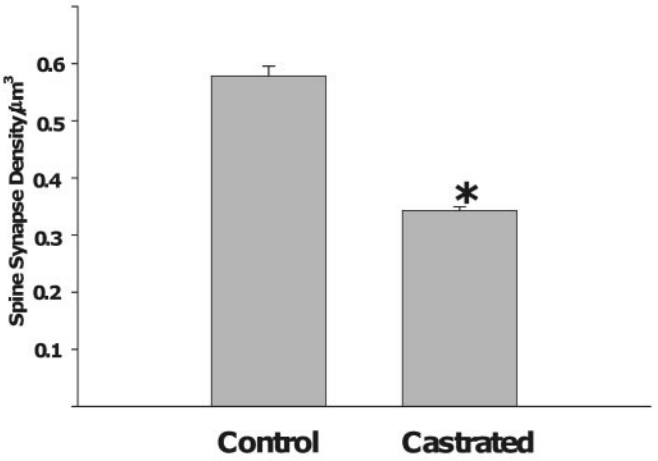

Figure 4. Bar graphs show a significant difference between the apical dendritic spine synapse densities of CA1 area pyramidal cells in the hippocampus of gonadectomized (1 month) and control monkeys. The spine synapse density in the control monkeys is 40% higher than in the castrated animals (mean ± SEM, $*P < 0.001$, $t = -12.817$, d.f. = 4).

tical analysis on the density of these processes and having the data of the light microscopic surface density calculation of glia processes (see below), an unbiased electron microscopic stereological calculation was not performed.

The result of the unbiased electron microscopic stereological calculation on the spine synapse density demonstrated a major, significant ($P < 0.001$) difference in the density values of CA1 area pyramidal cell spine synapse density of the two groups. The total numbers of spine synapses/*kv* counted in a 475 µm³ volume of control monkeys were 259.825, 287.802, and 276.45 (0.343 ± 0.00674/µm³). Whereas, in GDX animals, these numbers were 176.675, 156.75 and 163.875 (0.578 ± 0.0171/µm³), in the same volume (Table 1 and Fig. 4), which represents a 40% decrease.

Diameter of Dendritic Shafts

The semiquantitative analysis on the diameter of dendritic shafts in the outer third of the stratum radiatum of the CA1

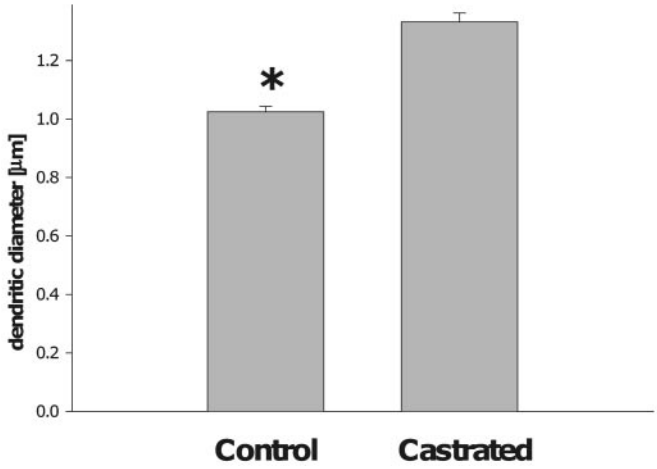

Figure 5. Bar graphs demonstrate a significant difference between the diameters of the apical dendrites in CA1 area pyramidal cells in the hippocampus of castrated (1 month) and control monkeys. The diameter in the castrated monkeys is 30% higher than in the control animals (mean ± SEM, $*P < 0.001$, $t = -8.776$, d.f. = 4).

subfield indicated that the average diameter of these dendrites is significantly ($P < 0.001$) larger in the GDX animals (1.332 ± 0.03 µm) than those (1.025 ± 0.018 µm) of control monkeys (Table 1 and Fig. 5). Thus, castration resulted in a 30% increase of the apical dendritic shaft diameter.

Discussion

Technical Notes

In spite of the 40% loss of spine synapses in the CA1 area of GDX monkeys, there was no apparent difference in the density of pyramidal and glia cells. Thus, a change in the volume of the hippocampus could be excluded. Two compensatory factors may be responsible for this phenomenon: the significant sprouting of glia processes and the significant, 30% enlargement of the diameter of pyramidal cell apical dendritic shafts. Since the increase in glia process density (15%) was much less than the decrease in the spine synapse density (40%), the remaining vacant space may have been filled up by the increased mass of dendrites. Enlargement of dendritic processes has been observed in female monkeys following OVX and this has been assumed to result from incorporation of the cytoplasm of spines that are retracted into the dendrites (Leranth *et al.*, 2002). The enlargement of dendritic processes seen here in male monkeys may be due to a similar spine retraction.

Functional Considerations

The major observation of this study is that, similar to gonadal hormone action on female rats (Gould *et al.*, 1990; Woolley *et al.*, 1990, 1997; Woolley and McEwen, 1992, 1993; Leranth and Shanabrough, 2001; Leranth *et al.*, 2000), female monkeys (Leranth *et al.*, 2002) and male rats (Leranth *et al.*, 2003), experimental manipulation of gonadal hormone levels greatly influences the density of pyramidal cell spine synapses in the CA1 hippocampal subfield of male subhuman primates. Thus, it can be assumed that the male primate hippocampus, in addition to other gonadal hormone-targeted structures that have major roles in mnemonic processes, e.g. the mesencephalic

dopamine system in conjunction with the prefrontal cortex (McCarthy *et al.*, 1996; Goldman-Rakic, 1998), is a major participant in the system by which gonadal hormones influence mnemonic functions. However, the specific mnemonic consequences of the observed spine synapse density changes in male monkeys are unclear at the present time. In conjunction with the estrogenic facilitation of hippocampal LTP and neurogenesis seen in female rats (e.g. Wong and Moss, 1992; Warren *et al.*, 1995; Tanapat *et al.*, 1999), it has been assumed that the increased spine density reported during proestrus in cycling rats or in ovariectomized rats after exogenous estrogen should lead to improvements in learning and memory. Indeed, increased spine density in the CA1 region of the hippocampus has been associated with spatial learning (Moser *et al.*, 1994, 1997). However, studies in intact cycling rats have failed to find fluctuations in spatial memory that mirror changes in spine density (Frye, 1995; Berry *et al.*, 1997; Stackman *et al.*, 1997; Warren and Juraska, 1997). Furthermore in male rats, exogenous testosterone has been shown to impair the acquisition of a spatial water maze task (Goudsmit *et al.*, 1990; Naghdi *et al.*, 2001). Nevertheless, several studies in humans have demonstrated a positive relationship between testosterone levels and several types of memory, particularly in older men (Gouchie and Kimura, 1991; Janowsky *et al.*, 1994, 2001; Barrett-Connor *et al.*, 1999; Silverman *et al.*, 1999; Cherrier *et al.*, 2001; Moffat *et al.*, 2002). In several cases, men with lower T levels exhibited impaired memory relative to those with higher T (Barrett-Connor *et al.*, 1999; Moffat *et al.*, 2002). Because the cytoarchitecture is similar between humans and monkeys (e.g. Rosene and Van Hoesen, 1987), it is possible that humans with low testosterone may have lower spine densities.

Sexual Differences

Data from previous experiments regarding the response of the pyramidal cell spine synapse density to the manipulation of gonadal hormone levels of male (Leranth *et al.*, 2003) and female (Leranth *et al.*, 2000) rats, as well as female (Leranth *et al.*, 2002) and male monkeys can be directly compared. In rats, similar to monkeys, experimental manipulations of circulating gonadal hormone levels have no effect on pyramidal cell density in either female or male animals. In contrast, gonadectomy has a significant effect on the density of the dendritic spine synapses of pyramidal cells in both female and male rats, as well as female and male monkeys (Table 2).

From this table, it appears that, in percentage terms, the magnitude of the synaptoplastic effect of female and male gonadal hormones is similar in rats (50%) and monkeys (40%) of both genders. The absolute spine synapse density values of control female rats (1.08/μm^3) and monkeys (1.15/μm^3) are also very similar. However, there are marked differences between the absolute values of spine synapse densities of control male rats (0.9/μm^3) and monkeys (0.58/μm^3). Consequently, there are major differences between the spine synapse density values of female and male monkeys regardless of the levels of circulating gonadal hormones. Both the control (0.58/μm^3) and GDX males (0.34/μm^3) have ~48% fewer spine synapses/μm^3 than control (1.15/μm^3) and OVX (0.65/μm^3) female monkeys, respectively. Furthermore, the spine synapse density in the intact, control male monkey hippocampus (0.58/μm^3) is >10% lower than in the OVX female monkey (0.65/μm^3).

In light of these data, assumptions regarding spine density and memory may need to be reevaluated or reconsidered. It is generally accepted that hippocampal long-term potentiation (LTP) is associated with synaptic plasticity and memory (Cohen and Eichenbaum, 1991) and electrophysiological experiments have demonstrated that E-induced spine synapse density in OVX rats is associated with augmented CA1 long-term potentiation (LTP; Cordoba Montoya and Carrer, 1997; Foy *et al.*, 1984; Good *et al.*, 1999; Ito *et al.*, 1999). In contrast, T administration to castrated male rats has been reported to have an opposite, negative effect on CA1 LTP (Harley *et al.*, 2000), in spite of the fact that T administration dramatically increases the spine synapse density in both male rats (50%; Leranth *et al.*, 2003) and monkeys (40%; recent study). However, sex differences favoring males have been observed in some hippocampal-dependent memory tasks (Williams *et al.*, 1990; Williams and Meck, 1991; Roof and Havens, 1992; Roof, 1993; Markowska, 1999). Exogenous T or E during early postnatal development can reverse this sex difference and promote the development of a male-like hippocampus in females (Williams *et al.*, 1990; Williams and Meck, 1991; Roof and Havens, 1992; Roof, 1993). Because males excel at some hippocampal-dependent memory tasks, despite having fewer CA1 spine synapses than females, it is tempting to speculate that either spine density has little to do with hippocampal-dependent memory processing, or that both LTP and gonadal hormones induce synaptic sprouting without causal relationships. On the other hand, the organizational effects of steroid hormones on the brain may necessitate that females have a greater density of CA1 spine synapses to compensate for spine reductions or other alterations elsewhere in the brain. Alternatively, high numbers of spine synapses may not be beneficial to memory (e.g. by producing excess disruptive input to the dendrite) and the impairments shown by females in some spatial memory tasks may be the result of too many spine synapses. Correlations between spine synapse density and memory measurements performed in the same animals will be necessary to clearly elucidate this issue.

Table 2

Summary of spine density values in the CA1 hippocampal subfield of gonadectomized and control female and male rats and monkeys

Animals	Gonadectomized	Control	Difference (%)	
Female rat	0.71/μm^3	1.08/μm^3	34	Leranth *et al.* (2000)
Male rat	0.45/μm^3	0.9/μm^3	50	Leranth *et al.* (2003)
Female monkey	0.65/μm^3	1.15/μm^3	40	Leranth *et al.* (2002)
Male monkey	0.34/μm^3	0.58/μm^3	40	Present study

Notes

The authors thank Dr Harold Behrman for his help with the statistical analyses, Dr Neil J. MacLusky for his helpful advice in writing the discussion and the staff of the St Kitts Biomedical Research Foundation for their assistance with the study, especially O'Neal Whatley, Wellington Sutton, Sean O'Loughlin, Ernell Nisbett, Franclin Conner, Ricaldo Pike and Kristin Atterbury. Grant sponsors: NIH, grant numbers: MH 60858 and NS 42644 to C.L. and MH-59847 and RR-14451 to T.L.H.; and Deutsche Forschungsgemeinsaft, grant number: PR703/1-1 to J.P-K.

Address correspondence to Csaba Leranth, Department of Obstetrics and Gynecology, Yale University, School of Medicine, 333 Cedar Street, FMB 312, New Haven, CT 06520-8063, USA. Email: csaba.leranth@yale.edu.

References

Abdelgadir SE, Roselli CE, Choate JV, Resko JA (1999) Androgen receptor messenger ribonucleic acid in brains and pituitaries of male rhesus monkeys; studies on distribution, hormonal control, and relationship to luteinizing hormone secretion. Biol Reprod 60:1251–1256.

Barrett-Connor E, Goodman-Gruen D, Patay B (1999) Endogenous sex hormones and cognitive function in older men. J Clin Endocrinol Metab 84:3681–3685.

Berry B, McMahan R, Gallagher M (1997) Spatial learning and memory at defined points of the estrous cycle: effects of performance of a hippocampal-dependent task. Behav Neurosci 111:267–274.

Braendgaar H, Gundersen HJG (1986) The impact of recent stereological advances on quantitative studies of the nervous system. Neurosci Methods 18:39–78.

Brown TJ, Sharma M, Karsan N, Walters MJ, MacLusky NJ (1995) *In vitro* autoradiographic measurement of gonadal steroid receptors in brain tissue sections. Steroids 60:726–737.

Cherrier MM, Asthana S, Plymate S, Baker L, Matsumoto AM, Peskind E, Raskind MA, Brodkin K, Bremner W, Petrova A, LaTendresses S, Craft S (2001) Testosterone supplementation improves spatial and verbal memory in healthy older men. Neurology 57:80–88.

Choate JV, Slayden OD, Resko JA (1998) Immunocytochemical localization of androgen receptors in brains of developing and adult male rhesus monkeys. Endocrine 8:51–60.

Clancy AN, Bonsall RW, Michael RP (1992) Immunohistochemical labeling of androgen receptors in the brain of rat and monkey. Life Sci 50:409–417.

Cohen NJ, Eichenbaum H (1991) The theory that wouldn't die: a critical look at the spatial mapping theory of hippocampal function. Hippocampus 1:265–268.

Cordoba Montoya, DA and Carrer, HF (1997) Estrogen facilitates induction of long term potentiation in the hippocampus of awake rats. Brain Res 778:430–438.

Foy MR, Chiaia NL, Teyler TJ (1984) Reversal of hippocampal sexual dimorphism by gonadal steroid manipulation. Brain Res 321:311–314.

Frye CA (1995) Estrus-associated decrements in a water maze task are limited to acquisition. Physiol Behav 57:5–14.

Goldman-Rakic PS (1998) The cortical dopamine system: role in memory and cognition. Adv Pharmacol 42:707–711.

Good M, Day M, Muir J (1999) Cyclical changes in endogenous levels of oestrogen modulate the induction of LTD and LTP in the hippocampal CA1 region. Eur J Neurosci 11:4476–4480.

Gouchie C, Kimura D (1991) The relationship between testosterone levels and cognitive ability patterns. Psychoneuroendocrinology 16:323–334.

Goudsmit E, Van de Poll NE, Swaab DF (1990) Testosterone fails to reverse spatial memory decline in aged rats and impairs retention in young and middle-aged animals. Behav Neural Biol 53:6–20.

Gould E, Woolley CS, Frankfurt M, McEwen BS (1990) Gonadal steroids regulate spine synapse density in hippocampal pyramidal cells in adulthood. J Neurosci 10:1286–1291.

Harley CW, Malsbury CW, Squires A, Brown RAM (2000) Testosterone decreases CA1 plasticity *in vivo* in gonadectomized male rats. Hippocampus 10:693–697.

Horvath TL, Diano S, van den Pol AN (1999) Synaptic interaction between hypocretin (Orexin) and neuropeptide Y cells in the rat and primate hypothalamus: a novel circuit implicated in metabolic and endocrine regulations. J Neurosci 19:1072–1087.

Ito K, Skinkle KL, Hicks TP (1999) Age-dependent, steroid-specific effects of oestrogen on long-term potentiation in rat hippocampal slices. J Physiol 515:209–220.

Janowsky JS, Oviatt SK, Orwoll ES (1994) Testosterone influences spatial cognition in older men. Behav Neurosci 108:325–332.

Janowsky JS, Chavez B, Orwoll E (2000) Sex steroids modify working memory. J Cogn Neurosci 12:407–414.

Kerr JE, Allore RJ, Beck SG, Handa RJ (1995) Distribution and hormonal regulation of androgen receptor (AR) and AR messenger ribonucleic acid in the rat hippocampus. Endocrinology 136:3213–3221.

Lam T-T, Leranth C (2003) Gonadal hormones act extrinsic to the hippocampus to influence the density of hippocampal astroglial processes. Neuroscience 116:491–498.

Leranth C, Shanabrough M (2001) Supramammillary area mediates subcortical estrogenic action on hippocampal synaptic plasticity. Exp Neurol 167:445–450.

Leranth C, Shanabrough M, Horvath TL (2000) Hormonal regulation of hippocampal spine synapse density involves subcortical mediation. Neuroscience 101:349–356.

Leranth C, Shanabrough M, Redmond DE, Jr (2002) Gonadal hormones are responsible for maintaining the integrity of spine synapses in the CA1 hippocampal subfield of female non-human primates. J Comp Neurol 447:34–42.

Leranth C, Petnehazy O, MacLusky NJ (2003) Gonadal hormones affect spine synaptic density in the CA1 hippocampal subfield of male rats. J Neurosci 23:1588–1592.

McCarthy G, Puce A, Constable RT, Krystal JH, Gore JC, Goldman-Rakic PS (1996) Activation of human prefrontal cortex during spatial and non-spatial working memory tasks measured by function of MRI. Cereb Cortex 6:600–611.

MacLusky NJ, Walters MJ, Clark AS, Toran-Allerand CD (1994) Aromatase in the cerebral cortex, hippocampus, and mid-brain: ontogeny and developmental implications. Mol Cell Neurosci 5:691–698.

Markowska AL (1999) Sex dimorphisms in the rate of age-related decline in spatial memory: relevance to alterations in the estrous cycle. J Neurosci 19:8122–8133.

Moffat SD, Zonderman AB, Metter EJ, Blackman MR, Harman SM, Resnick SM (2002) Longitudinal assessment of serum free testosterone concentration predicts memory performance and cognitive status in elderly men. J Clin Endocrinol Metab 87:5001–5007.

Moser B, Trommald M, Andersen P (1994) An increase in dendritic spine density on hippocampal CA1 pyramidal cells following spatial learning in adult rats suggests the formation of new synapses. Proc Natl Acad Sci USA 91:12673–12675.

Moser M, Trommald M, Egeland T, Andersen P (1997) Spatial training in a complex environment and isolation alter the spine distribution differently in rat CA1 pyramidal cells. J Comp Neurol 380:373–381.

Naghdi N, Nafisy N, Majlessi N (2001) The effects of intrahippocampal testosterone and flutamide on spatial localization in the Morris water maze. Brain Res 897:44–51.

Pomerantz SM, Sholl SA (1987) Analysis of sex and regional differences in androgen receptors in fetal rhesus monkey brain. Brain Res 433:151–154.

Roof RL (1993) Neonatal exogenous testosterone modifies sex difference in radial arm and Morris water maze performance in prepubescent and adult rats. Behav Brain Res 53:1–10.

Roof RL, Havens MD (1992) Testosterone improves maze performance and induces development of a male hippocampus in females. Brain Res 572:310–313.

Roselli CE, Klosterman S, Resko JA (2001) Anatomic relationships between aromatase and androgen receptor mRNA expression in the hypothalamus and amygdala of adult male cynomolgus monkeys. J Comp Neurol 439:208–223.

Rosene DL, Van Hoesen GW (1987) The hippocampal formation of the primate brain. A review of some comparative aspects of cytoarchitecture and connections. Cereb Cortex 6:345–456.

Rusakov DA, Richter-Levin G, Stewart MG, Bliss TV (1997) Reduction in spine density associated with long-term potentiation in the dentate gyrus suggests a spine fusion-and-branching model of potentiation. Hippocampus 7:489–500.

Silverman I, Kastuk D, Choi J, Phillips K (1999) Testosterone levels and spatial ability in men. Psychoneuroendocrinology 24:813–822.

Simerly RB, Chang C, Muramatsu M, Swanson LW (1990) Distribution of androgen and estrogen receptor mRNA-containing cells in the

rat brain: and *in situ* hybridization study. J Comp Neurol 294:76–95.

Small JV (1968) Measurement of section thickness. In: Proceedings of the European Conference on Electron Microscopy. Rome: Tipographia Poliglotta Vatican, pp. 609–610.

Stackman RW, Blasberg ME, Langan CJ, Clark AS (1997) Stability of spatial working memory across the estrous cycle of Long–Evans rats. Neurobiol Learn Mem 67:167–171.

Tanapat P, Hastings NB, Reeves AJ, Gould E (1999) Estrogen stimulates a transient increase in the number of new neurons in the dentate gyrus of the adult female rat. J Neurosci 19:5792–5801.

Warren SG, Juraska JM (1997) Spatial and non-spatial learning across the rat estrous cycle. Behav Neurosci 111:259–266.

Warren SG, Humphreys AG, Juraska JM, Greenough WT (1995) LTP varies across the estrous cycle: enhanced synaptic plasticity in proestrus rats. Brain Res 703:26–30.

Williams C, Meck WH (1991) The organizational effects of gonadal steroids on sexually dimorphic spatial ability. Psychoneuroendocrinology 16:155–176.

Williams C, Barnett AM, Meck WH (1990) Organizational effects of early gonadal secretions on sexual differentiation in spatial memory. Behav Neurosci 104:84–97.

Woolley CS, McEwen BS (1992) Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat. J Neurosci 12:2549–2554.

Woolley CS, McEwen BS (1993) Roles of estradiol and progesterone in regulation of hippocampal spine density during the estrus cycle in rat. J Comp Neurol 336:293–306.

Woolley CS, Gould E, Frankfurt M, McEwen BS (1990) Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. J Neurosci 10:4035–4039.

Woolley CS, Weiland NG, McEwen BS, Schwartzkroin PA (1997) Estradiol increases the sensitivity of hippocampal CA1 pyramidal cells to NMDA receptor-mediated synaptic input: correlation with dendritic spine density. J Neurosci 17:1848–1859.

Wong M, Moss RL (1992) Long-term and short-term electrophysiological effects of estrogen on the synaptic properties of hippocampal CA1 neurons. J Neurosci 12:3217–3225.