

Sexually selected size differences and conserved sexual monomorphism of genital cortex

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Abstract

The mammalian somatosensory cortex shows marked species-specific differences. How evolution in general and sexual selection in particular shape the somatosensory cortical body representation has not been delineated, however. Here we address this issue by a comparative analysis of genital cortex. Genitals are unique body parts in that they show sexual dimorphism, major changes in puberty and typically more pronounced species differences than other body parts (Hosken & Stockley, 2004). To study the evolution of genital cortex we flattened cortical hemispheres and assembled 104 complete body maps, revealed by cytochrome-oxidase activity in layer 4 of 8 rodent and 1 lagomorph species. In two species, we also performed antibody stainings against vesicular glutamate transporter-2, which suggested that cytochrome-oxidase maps closely mirror thalamic innervation. We consistently observed a protrusion between hindlimb and forelimb representation, which in rats (Lenschow et al., 2016) corresponds to the penis representation in males and the clitoris representation in females. Consistent with the idea that this protrusion corresponds to genital cortex, we observed a size increase of this protrusion during puberty. Species differed in external genital sexual dimorphism, but we observed a sexual monomorphism of the putative genital protrusion in all species, similar to previous observations in rats. The relative size of the putative genital protrusion varied more than 3-fold between species ranging from 0.5% of somatosensory cortex area in chipmunks to 1.7% in rats. This relative size of the genital protrusion co-varied with relative testicle size, an indicator of sperm competition and sexual selection.

KEYWORDS

clitoris, genitals, penis, RRID: AB_2187552, RRID: AB_141607, sexual selection, somatosensory cortex

1 | INTRODUCTION

Primary sensory cortical areas show both species differences and conserved features, such as topographic representation of the sensory surface (Krubitzer & Kaas, 2005). Also the internal organization of sensory areas shows similarities: In case of the primary somatosensory cortex (S1), one observes a medial localization of the representation of trunk and limbs and a more lateral localization of the representation of the head. Species also show clear differences in areal size, shape, and modularity in cortical fields. Variations in relative size exist between and within sensory systems and reflect specializations to specific ecological niches. Diurnal squirrels, for example, have a large visual cortex and a small somatosensory cortex, whereas nocturnal muroid rodents show a greatly expanded somatosensory cortex, characterized by a dominant

representation of the vibrissae (Krubitzer, 1995). Further, it was found that raccoons, which show elaborate manual abilities, shows a greatly enlarged paw representation (Welker & Seidenstein, 1959). In many instances, however, one cannot pinpoint the evolutionary forces that shape the patterning of somatosensory cortex. For example, cortical barrels are some of the most prominent and most investigated cortical structures, but their occurrence across species shows no obvious link to either life style or taxonomy (Woolsey, Welker, & Schwartz, 1975).

Here we investigate evolutionary patterns of the genital representation in somatosensory cortex. It has long been recognized that external genitals show a high degree of divergence, even among closely related taxa (Hosken & Stockley, 2004) and scale weakly with body mass (Ramm, 2007). Initially it was suggested (Eberhard, 1985) that genital divergence might function as a lock and key mechanism

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preventing hybridization. Comparative studies did not support this idea, however, and current thinking favors other evolutionary mechanism such as cryptic female choice or sexually antagonistic coevolution (Eberhard, 2010). While mechanisms of selection are not entirely resolved, divergence in insects was found to be higher in polyandrous species compared to monandrous species (Hosken & Stockley, 2004). Furthermore, relative baculum length co-varies with relative testis mass in rodents and carnivores, a reliable indicator for sperm competition. Additionally, it has been suggested that a larger baculum and glans penis has positive effects on postcopulatory fertilization success (Ramm, 2007).

While the evolutionary mechanisms driving genital evolution are debated, there is almost no information available about the evolution of the neural control structures that process genital information. The role of brain structures in sexual selection has been discussed in a variety of contexts. Different theories, such as the sensory bias model (Endler, 1992) or the sensory exploitation model (Ryan, 1990) try to address this issue, mostly concerning the auditory, olfactory, and visual system. The authors discuss adaptations of male signals to stimulate the sensory system of the female to enhance their chance for copulation and the role of sexual selection on the evolution of the sensory system itself.

The recent identification of a large, anatomically identifiable, genital representation (Lenschow et al., 2016) in the rat somatosensory cortex opens up an avenue to study the evolution of the neural representation of genital signals. Contrary to the external dimorphism of male and female genitals, the cortical representation was monomorphic. Furthermore, Lenschow et al. (2016) observed a high degree of plasticity of the genital cortex during puberty, showing greatly increased areal growth compared to the rest of somatosensory cortex.

In this study, we address following questions using a comparative approach:

1. Is the distinctive representation of penis/clitoris conserved across species?
2. Is genital cortex sexually monomorphic across species?
3. Can growth of genital cortex be observed in other species?
4. Is there a correlation between socio-sexual behavior and genital representation?

To answer these questions, we compare cytochrome-oxidase staining of flattened cortices in nine species (Mouse, Rat, Mongolian Gerbil, Syrian Hamster, Degu, Guinea Pig, American Chipmunk, Chinchilla, Rabbit) and analyzed the brains by reconstructions of the full body map through serial sections.

2 | MATERIALS AND METHODS

2.1 | Animals

All experimental procedures were performed according to German guidelines on animal welfare under the supervision of local ethics committees. Animals were killed according to the permit (T0230/15). Ham-

sters and mice were purchased from Janvier Labs (St Berthevin Cedex, France). New Zealand white rabbits and guinea pigs were purchased from Envigo (An Venray, the Netherlands). Chinchillas were purchased from Moulton's Chinchilla ranch (Rochester, MN, USA). Degus were provided by Leibniz Institute for Neurobiology (Magdeburg, Germany). Eastern Chipmunks were bought from Exotic Farm (Buxheim, Germany). Data for rats is adapted from Lenschow et al. (2016). Brains of male/female and prepubertal/adult animals have been analyzed (Table 1).

2.2 | Histology

Animals received an overdose of the anesthetic (20% urethane solution, 1 ml/100 g). External genitalia were photographed and measured. Subsequently, the animals were transcardially perfused with phosphate buffer followed by a 2% paraformaldehyde (PFA) solution. The low PFA concentration was chosen, in order to increase the contrast in cytochrome-oxidase-activity signals. Brains were removed (Figure 1a), hemispheres were separated and subcortical structures detached. Cortices were flattened in phosphate buffer between two glass slides separated by clay spacers. Glass slides were weighed down with small ceramic weights for ~3–5h at 4°C (Figure 1b). Flattened cortices (Figure 1c) were then stored overnight in a 1 or 2% PFA solution, whereby the best results were obtained using 1% PFA for postfixation. Hemispheres were cut into 80 µm sections on a Vibratome (Microm HM 650V, Thermo Scientific). Sections were stained for cytochrome-oxidase activity (Figure 1d) using the Wong-Riley (1989) protocol at first and later on the Divac, Mojsilovic-Petrovic, López-Figueroa, Petrovic-Minic, and Møller (1995) protocol. The Wong-Riley (1989) protocol mostly lead to insufficient staining contrast in areas with lower cytochrome-oxidase activity, such as trunk, fore, and hindpaw representation. Hence, the Divac et al. metal ions protocol (1995) was used for subsequent animals. As a consequence, rat (prepubertal & adult) and mouse (prepubertal) brains were stained using the Wong-Riley (1989) protocol and all further tissues were stained using the Divac et al. (1995) protocol.

The incubation solution after Wong-Riley (1989) consisted of: cytochrome c type III (80 µM), 3–3'-diaminobenzidine tetrahydrochloride (DAB, 1.56 mM), phosphate buffer (PB, 0.1 M) and Catalase (83.3 nM). The incubation solution after Divac et al. (1995) consisted of: cytochrome c type III (16.15 µM), 3–3'-diaminobenzidine tetrahydrochloride (DAB, 2.8 mM), Hepes buffer (0.1 M), nickel ammonium sulfate (NiAS, 3.165 mM) and sucrose (117 mM).

After the staining procedure, sections were mounted on gelatin coated glass slides with Mowiol® mounting medium. Mounted sections were kept in the refrigerator to avoid bleaching. Subsequently, pictures were taken on a microscope (Olympus BX51) using the virtual tissue function. In one case, it was necessary to align and stitch images together from two sections to show the genital region in a complete and intelligible way. Pictures were adjusted with brightness, contrast and HDR toning.

VGLuT2 immunohistochemical labeling was performed using standard procedures. Brain sections were pre-incubated for an hour at room

TABLE 1 Summary of animals

Species/strain	Condition	No. of animals (male/female)	Age (weeks)	Supplier
Rat (<i>Rattus norvegicus</i> , RJHan:WI)	Prepubertal	3/3	2	Janvier
	Adult	3/3	6/8	
Mouse (<i>Mus musculus f. domestica</i> , C57BL/6JR)	Prepubertal	2/3	2	Janvier
	Adult	3/2	6	
Gerbil (<i>Meriones unguiculatus</i> , RJTub:Mon)	Prepubertal	1/2	2	Janvier
	Adult	3/3	8	
Hamster (<i>Mesocricetus auratus</i> , RJHan:AURA)	Prepubertal	2/2	2	Janvier
	Adult	2/3	9	
Rabbit (<i>Oryctolagus cuniculus f. domestica</i> , HsdJf:NZW)	Prepubertal	2/2	4	Envigo
	Adult	1/1	22	
Guinea pig (<i>Cavia porcellus</i> , HsdDhl:DH)	Adult	2/2	10	Envigo
Chipmunk (<i>Tamias striatus</i>)	Adult	2/2	20	Exotic Farm
Chinchilla (<i>Chinchilla lanigera</i>)	Adult	2/2	40	Moulton's Chinchilla Ranch
Degu (<i>Octodon degus</i>)	Adult	2/2	20	Leibniz Institute for Neurobiology

temperature in a blocking solution (0.1 M PBS, 2% Bovine Serum Albumin and 0.5% Triton X-100). Afterwards, primary antibodies were diluted in a solution containing 0.5% Triton X-100 and 1% Bovine

Serum Albumin. The primary antibody against VGluT2 (Millipore Cat# MAB5504 Lot# RRID:AB_2187552) was incubated with the free-floating sections for at least 24 hr under mild shaking at 4°C. Incubation

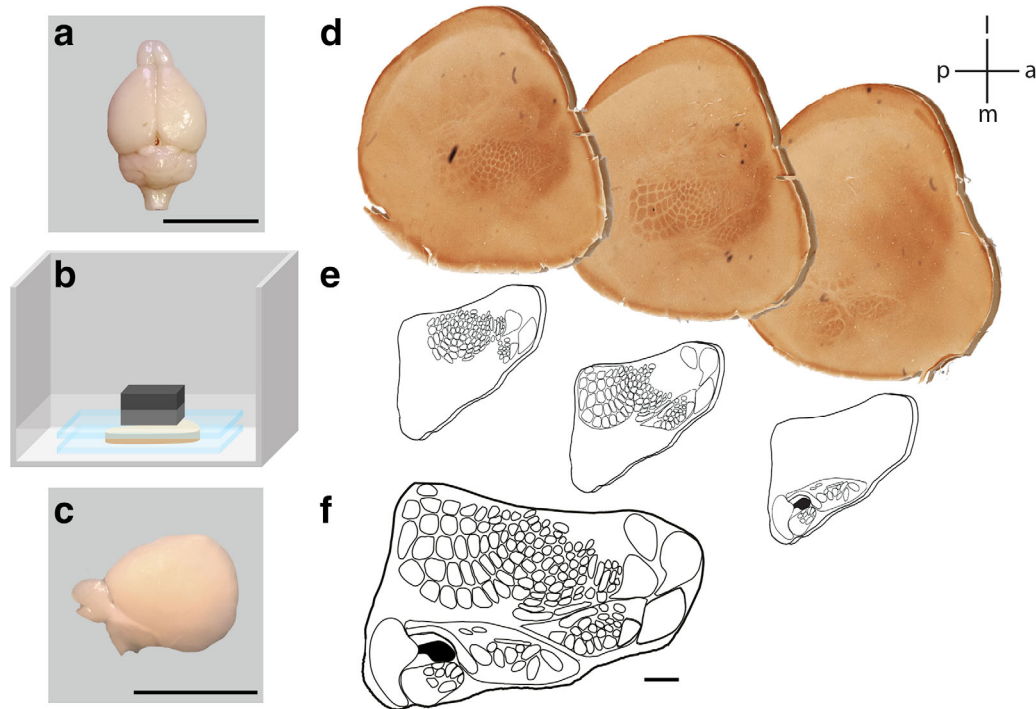


FIGURE 1 Flattening of cortical hemispheres, cytochrome oxidase staining and reconstruction of the body representation through serial sections. (a) Brain of a mouse. (scale bar = 1 cm). (b) Subcortical structures were removed and cortices were flattened between two glass slides in phosphate buffer. (c) Flattened hemisphere of a. (scale bar = 1 cm). (d) Hemispheres were tangentially sectioned and stained for cytochrome oxidase activity (dark precipitate). a: anterior; p: posterior; l: lateral; m: medial. (e) Granular somatosensory regions (dark precipitate) were reconstructed for each section. (f) Serial reconstruction was merged into a complete body map of the somatosensory cortex (scale bar = 500 μ m). Genital cortex is highlighted in black

with the primary antibody was followed by detection with a secondary antibody coupled to the fluorophore Alexa 488 (Molecular Probes Cat# A-21202 also A21202 Lot# RRID:AB_141607). The secondary antibody was diluted (1:500) in 0.5% Triton X-100 and the reaction was allowed to proceed for 2 hr in the dark at room temperature. Sections were mounted on gelatin coated glass slides with Fluoromount® (Biozol, Eiching, Germany) mounting medium. Pictures were taken on a Leica DM5500B and subsequently adjusted with brightness and contrast.

2.3 | Quantification of somatosensory areas and sizes

The outlines of cortical granular layer 4 regions, indicated by a dark precipitate from the cytochrome-oxidase stain, were drawn with Neurolucida (MicroBrightField, Colchester, USA) (Figure 1e). Merged body maps were reconstructed through serial sections (Figure 1f). The area of various somatosensory regions was calculated using Neurolucida area calculating tool. The area of the following cortical representations was measured: hindpaw, forepaw, trunk, interlimb cortex, and genital. The fraction of genital cortex of the whole S1 area was calculated by dividing the genital area by the value of the S1 area. All statistic tests were conducted in Matlab (The MathWorks Inc., Natick, USA)/Excel (Microsoft Corporation, Redmond, USA).

2.4 | Comparison of relative genital cortex size and sperm competition markers

The length of the external genital was measured in males and females for every species investigated. Body length was measured from nose to base of the tail. Testicle mass was taken from the literature (guinea pig and rabbit [Matousek, 1969]; mouse [Chamindrani Mendis-Handagama, Siril Ariyaratne, Fecteau, Grizzle, & Jayasundera, 2010]; gerbil [Ninomiya & Nakamura, 1987]; hamster [Siegel, 1985], degu [Obregón & Ramirez, 1997]; rat [de Souza Predes, Diamante, & Dolder, 2010]; chinchilla [de Soya, 2007]). As measurements for body mass, average weight on week of maturation was used (data provided by breeder). Relative genital cortex length was acquired by comparing length of genital cortex to length of body representation (ear representation to medial end of trunk representation).

3 | RESULTS

3.1 | Identification of genital cortex in mammalian brains

As detailed in the methods section, we derived a large number of somatosensory cortex body maps in various species (Table 1). We combined cytochrome-oxidase staining (Figure 2a-f, top) and anatomical reconstruction from serial sections of the somatosensory cortices from a variety of species (Figure 2a-f, bottom) as previously done in the rat (Lenschow et al., 2016). The representation of the genitals is located anterior and medial to the posteromedial barrel subfield, which represent the macrovibrissae and is a prominent landmark in most cortices. In accordance with the general somatotopy, genital cortex is anatomically attached to the trunk representation and separated from fore and hind-

paw representations by non-granular septa. With respect to the topographic body representation, it shows an erect posture (Lenschow et al., 2016). Taking these criteria into account, we were able to anatomically identify the genital representation in eight further mammals: mouse (Figure 2b), gerbil (Figure 2c), hamster (Figure 2d), guinea pig, chipmunk, degu (Figure 2e), chinchilla (Figure 2f), rabbit (top, cytochrome-oxidase staining; bottom, reconstructed body map of the regions of interest). It was often necessary to reconstruct the trunk/genital area through multiple sections, because—as a result of the very medial position—this part of the somatosensory cortex often bends down medially out of the plane of the sections. Similar to the observation in rats, genital cortex was often divided from the forelimb and hindlimb by sharp septa with low cytochrome-oxidase activity. In some species (such as mice), the genital cortex had a higher cytochrome-oxidase activity than trunk cortex, showing a well-defined border at the base of the protrusion. In the absence of such a staining difference, the genital cortex was therefore defined in an analogous manner as the area, which protruded from the trunk representation. The staining of tangential sections of flattened cortices in rats (Figure 3a) and mice (Figure 3b) with vesicular glutamate transporter 2 (VGluT2) antibodies confirmed the observations of a distinct representation of the genitals (top: expression pattern of VGluT2; bottom: reconstructed body map of the regions of interest). Cytochrome-oxidase maps and VGluT2 staining led to similar map; as VGluT2 is highly expressed in thalamic terminals (Nahmani & Erisir, 2005) this observation suggests that genital maps mirror areas of thalamic innervation.

3.2 | Areal growth of genital cortex during puberty

Growth of genital cortex during puberty has been reported in the rat (Lenschow et al., 2016). The area of penis and clitoris representation as defined by the layer 4 cytochrome-oxidase activity showed morphological growth when comparing prepubertal to adult animals. This growth differed significantly to the total area growth of S1. We assessed body maps of prepubertal (left, P14 [rat, mouse, hamster]; P30 [rabbit]) and adult (right) mice, gerbils, hamsters, and rabbits (Figure 4a). Rat body maps came from Lenschow et al. (2016). The total area of S1 does not differ strongly between young and adult animals, whereas a large increase in genital cortex area is visible. Length and width increase equally, while general morphology is conserved. Pooled area measurements of S1 (Figure 4b) and genital cortex (Figure 4c) in prepubertal and adult animals allowed a striking observation: while the area of S1 changes little, the area of genital cortex increases by a larger factor. The scaling factors of S1 and genital cortex differ significantly (Figure 4d, $p = .002$, three-way ANOVA) and range between 1 (hamster) and 1.47 (rabbit), whereas scaling factors for genital cortex range between 1.38 (mouse, female) and 3.04 (gerbil, male). Scaling factors do not differ between sex (S1: $p = .43$, genital cortex: $p = .12$, three-way ANOVA).

3.3 | Monomorphic cortical representation of penis and clitoris

Lenschow et al. (2016) reported a cortical monomorphism of penis and clitoris representation in the rat. This result is surprising, given the

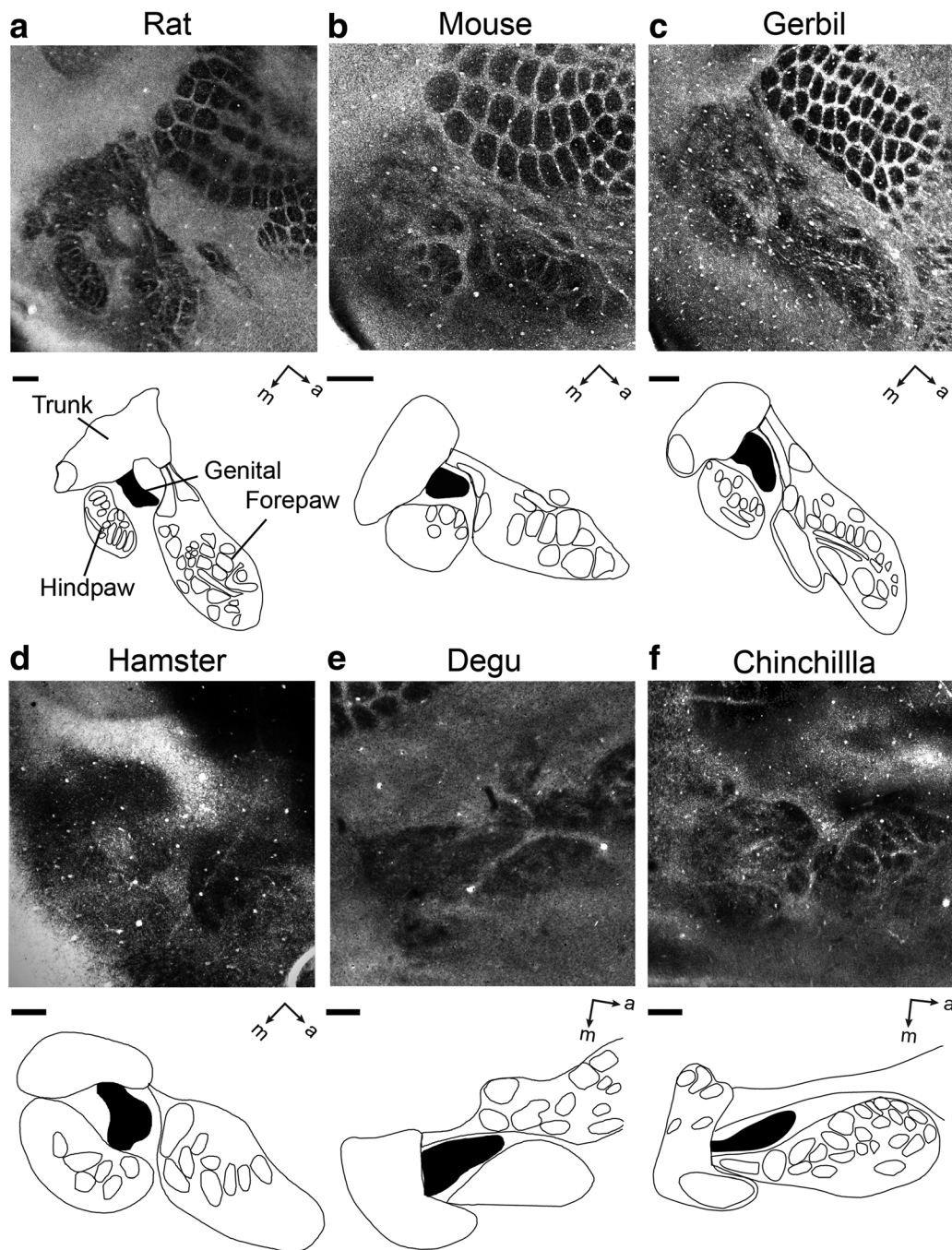


FIGURE 2 Identification of the putative genital representation across species. (a) Top: Tangential section of somatosensory cortex from a male rat stained for cytochrome oxidase activity (adapted from Lenschow and Brecht, in revision). Bottom: Merged serial reconstruction of medial portion of body map (scale bar = 500 μ m; black: genital cortex; adapted from Lenschow and Brecht, in revision). (b) Same as (a), but for a male mouse, aged P14. (c) Same as (a), but for an adult male gerbil. (d) Same as (a), but for an adult female hamster. (e) Same as (a), but for an adult male degu (stitched together from two aligned sections). (f) Same as (a), but for an adult female chinchilla

marked sexual dimorphism of rat external genitals. To assess if cortical sexual monomorphism is unique to rats, we compared the external genital size and cortical representation across species. The sexual dimorphism of penis and clitoris varied markedly across species (Figure 5). In some species, such as chinchillas (Figure 5a), we observed pronounced length difference between penis and clitoris, whereas this difference was less pronounced in other species, such as the rabbit

(Figure 5b). In all species, the penis was bigger than the external clitoris, also shown for the male (left) and female (right) degu (Figure 6a). In contrast, cortical representation of penis and clitoris are monomorphic in the species investigated as shown for male (left) and female (right) degu, gerbil, guinea pig, and chipmunk (Figure 6b). Across animals, length measurements showed significantly longer penises than external clitorises (Figure 6c, $p = .008$, paired t test), the largest differences

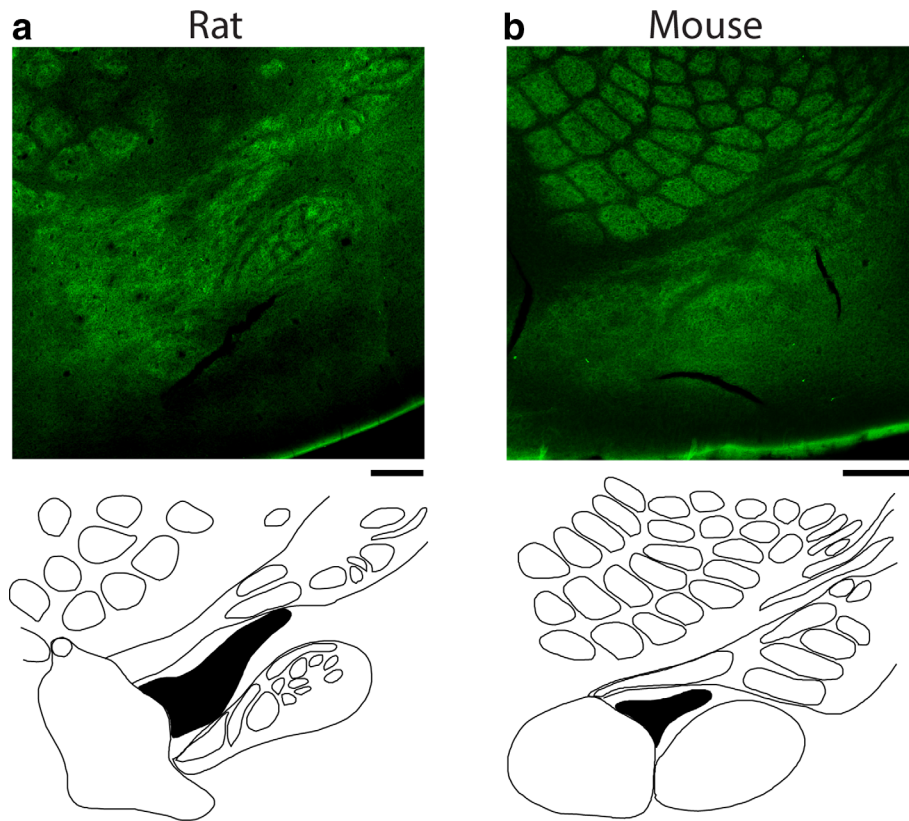


FIGURE 3 Visualization of the putative genital representation by VGLUT2 antibodies. (a) Top: Immunohistochemical localization of VGLUT2 (vesicular glutamate transporter 2) in tangential section of somatosensory cortex from a female rat, aged P42. Bottom: Merged serial reconstruction of medial portion of body map (scale bar = 500 μ m; black: genital cortex). (b) Same as (a), but for a male mouse, aged P14

were found in guinea pig (penis/clitoris factor: 4.3) and chinchilla (penis/clitoris factor: 3.8); smallest differences in rabbit (penis/clitoris factor: 1.7) and degu (penis/clitoris factor: 1.6). In contrast, pooled measurements of the length (Figure 6d, $p = .99$, paired t test) and the area (Figure 6e, $p = 0.052$, paired t test) of the cortical penis and clitoris representation were very similar, showing a significantly smaller ratio of penis to clitoris in cortex, compared to external genital length (Figure 6f, $p = 0.008$, paired t test). This suggests that cortical monomorphism of genital representation is conserved across these species.

3.4 | Species differences in genital representation

Body size differences were accompanied by different cortex sizes and in turn different sizes of S1 (Figure 7a). To investigate, whether the proportion and shape of the genital cortex in S1 is the same or different in the species, body maps were normalized to the adult rat's body map (Figure 7b). Trunk and penis representation of male guinea pig, mouse, gerbil, hamster, and chipmunk were isolated in order to compare their relative morphology, posture, and size (Figure 7c). We observed a morphological diversity between individual animals and species. Shape and position relative to the trunk were consistent within the species. The comparison showed a morphology, which resembled to the erect external genital with respect to general somatotopy. The only exception was the non-erect genital representation of the chipmunk. The relative size of the penis representation differed strongly across species. The biggest

proportion of genital cortex (mean male area \pm SEM vs. mean female area \pm SEM; in %) in S1 was observed in rat ($1.71 \pm 0.09\%$ vs. $1.73 \pm 0.14\%$ of S1 area), hamster ($1.55 \pm 0.09\%$ vs. $1.25 \pm 0.039\%$ of S1 area) and guinea pig ($1.56 \pm 0.1\%$ vs. $1.46 \pm 0.04\%$ of S1 area).

We next analyzed, if the differences in relative area of genital cortex correlates with general markers for sperm competition, as relative penis length and relative testicle mass. The measured penis length was set in relation to the body length (nose to base of tail) and compared to the relative cortical penis length (ear barrels to medial end of trunk representation, Figure 7d). Testicle mass (see methods, values obtained from the literature) was set in relation to the body weight (average weight at onset of adulthood) and compared to the relative cortical penis area (Figure 7e). Strikingly, both comparisons were highly correlated. Species with a small relative penis length, like rabbit or mouse, showed a relatively short cortical penis representation. Species with a large relative penis length, like rat or hamster, had a relatively long cortical penis representation. The correlation was significant ($\rho = 0.80$; $p = .021$, Spearman's rank order correlation). Guinea pig and chinchilla did not follow the general trend, with a large relative penis length, but a small average relative cortical penis length. Comparison of the relative testicle mass showed similar results. Chinchilla and degu, which had a small relative testicle mass, also had a small relative cortical penis area. Rat and hamster, which had a big relative testicle mass, also had the biggest relative cortical penis area. Also this correlation was significant ($\rho = 0.76$; $p = .036$; Spearman's

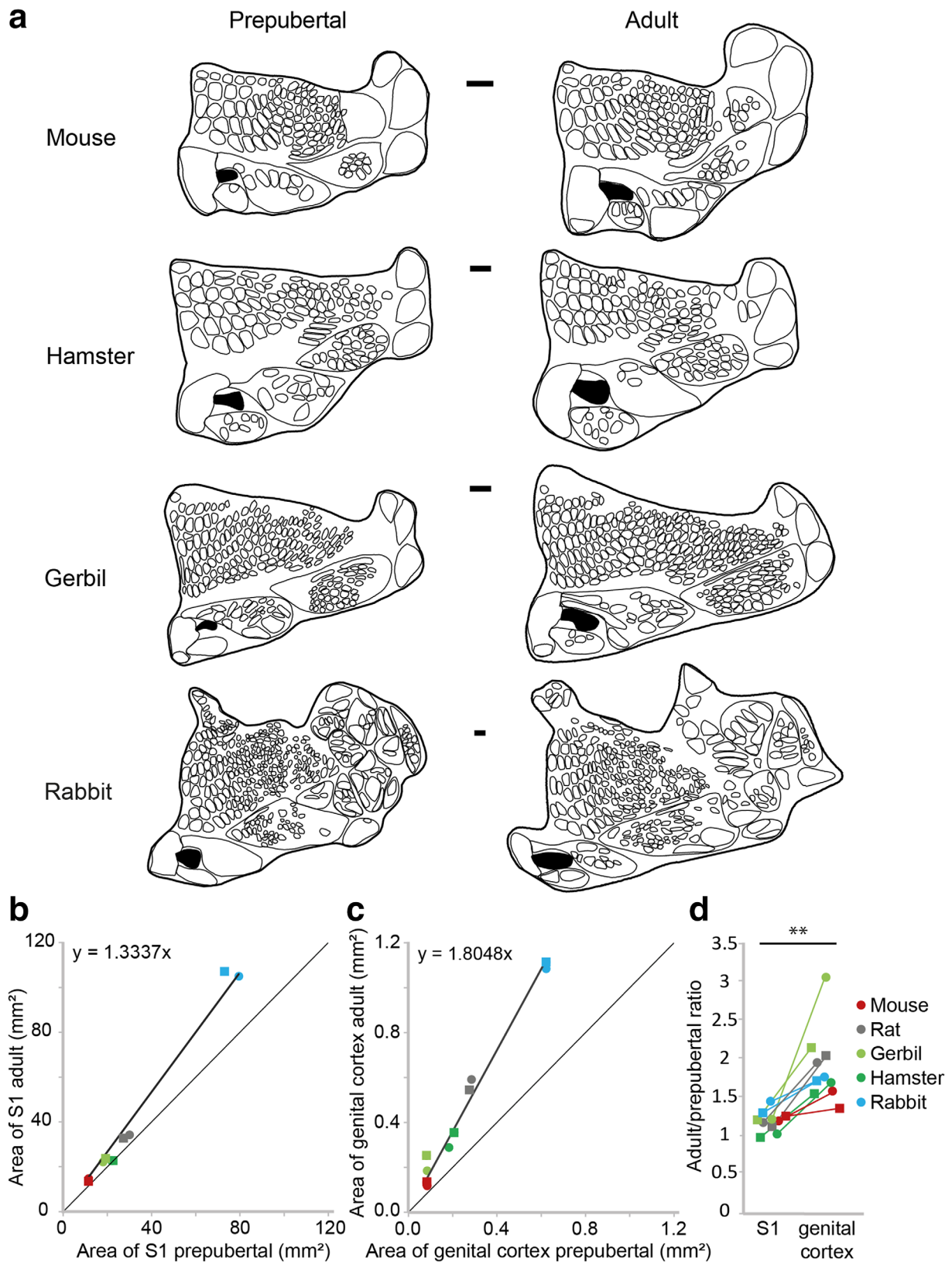


FIGURE 4 Genital cortex grows during puberty. (a) Body maps of male pup (left) and adult (right) mouse, gerbil, hamster, and rabbit (scale bar = 500 μ m; black: genital cortex). (b) Total area of somatosensory cortex (S1) of pups versus adults from different species. Square: female; circle: male. Data are fitted with line. (c) Same as (b), but for genital cortex area. (d) Ratios of adult versus young for S1 and genital cortex ($p = .002$, three-way ANOVA). Ratios varied for each species

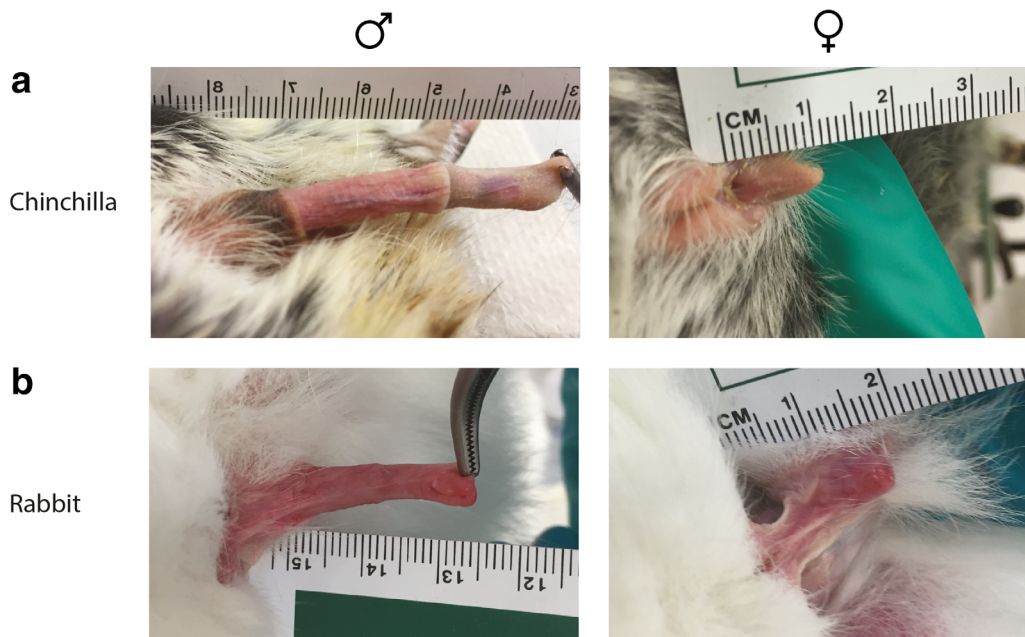


FIGURE 5 Varying degrees of genital dimorphism. (a) Strong sexual dimorphism of penis (left) and clitoris (right) in adult chinchillas (ruler scale = mm). (b) Modest sexual dimorphism of penis (left) and clitoris (right) in adult rabbits (ruler scale = mm)

rank order correlation). Thus, the relative size and length of the cortical penis representation correlate with the relative testicle size, a classic indicator of sperm competition.

4 | DISCUSSION

We identified the putative genital cortex in nine mammalian species. In all species studied, genital cortex was observed at a somatotopically appropriate location and had a similar size in both sexes. The comparison of prepubertal and adult animals in five species, support previous findings of pubertal growth of genital cortex, which greatly exceeds the relatively minor growth of S1 during puberty. Finally, comparison of relative genital cortex area of male animals among species revealed a strong correlation to markers of sexual selection, such as relative testicle size and relative penis length. This finding suggests that sexual selection shapes genital cortex size.

4.1 | Identification and morphology of genital cortex

We identified a putative genital cortex protrusion in nine mammalian species. Four observations suggest that this protrusion indeed represents genital cortex: (a) this protrusion was identified by Lenschow et al. (2016) with physiological methods as the penis and clitoris representation in male and female rats, respectively. (b) This protrusion is at the somatotopically appropriate position for penis and clitoris, respectively, i.e., it is in the body reference frame ventral from the trunk representation, ventral/anterior from tail and hindlimb representations and is posterior from the forelimb representation. (c) There are no other candidate body parts, which could be represented by this protrusion. (d) As discussed below, the size of the protrusion co-varies with markers of sexual selection. Collectively, these observations give us

confidence that rodents and lagomorphs have a genital representation in somatosensory cortex. Unlike the rest of S1, the putative genital cortex protrusion greatly expands during puberty. Numerous previous mapping studies failed to identify distinct representation of the genitals in the primary somatosensory cortex (Welker, 1971; Welker, 1976; Chapin & Lin, 1984; Gould, 1986; Dawson & Killackey, 1987; Rapisarda, Palmeri, Aicardi, & Sapienza, 1990; Catania & Remple, 2002; Krubitzer, Campi, & Cooke, 2011). We argue that the absence of genital responses in such studies reflects the low resolution of mapping experiments (typically in the several 100 μm range, whereas the cytochrome-oxidase maps provided here resolve detail in the 5–10 μm range) and perhaps experimenter biases against touching the genitals. We did not observe a distinct cytoarchitectonic module representing the testicles, but Lenschow et al. (2016) identified responses to scrotum stimulation adjacent to penis cortex. These more medial and posterior coordinates correspond somatotopically to a position in the lower ventral part of the trunk module of the layer 4 map; at these coordinates one observes responses to vulva stimulation in females. Our findings suggest that the representation of the genitals is conserved in rodents. Further studies should address, if genitals are represented distinctively in the Layer 4 body map of higher mammal orders and humans. Electrophysiological recordings in the macaque monkey have identified a distinct genital representation; laterally to the representation of the foot in areas 3b, 1, and 2 (Rothmund, Qi, Collins, & Kaas, 2002). Based on neural responses, the authors assumed a small representation and large receptive fields. However, older studies in macaques identified a genital representation medial or ventral to the foot representation (Woolsey, Marshall, & Bard, 1942; Whitsel, Dreyer, & Roppolo, 1971). Rothmund et al. (2002) suggest that the genitals are represented on both sides of the foot and leg representation. Similar findings in the human somatosensory cortex are still controversially

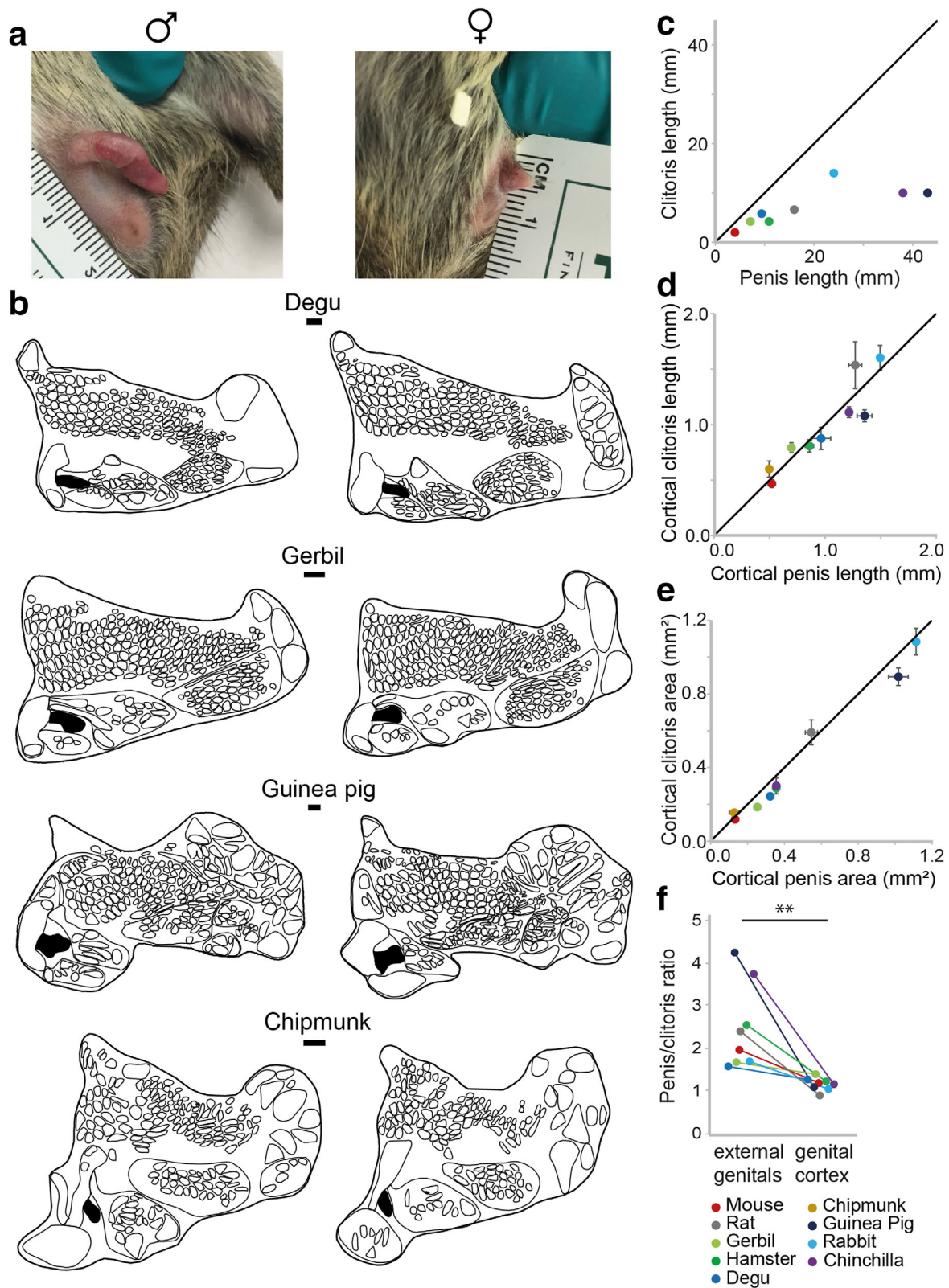


FIGURE 6 Representation of penis and clitoris is monomorphic across species. (a) External genital of male (left) and female (right) degu showed clear dimorphism in length (ruler scale = mm). This was observed in all other species (not shown). (b) Example maps for male (left) and female (right) degu, gerbil, guinea pig, and chipmunk (scale bar = 500 μ m; black: genital cortex). (c) Pooled measurements of external genital length revealed a pronounced sexual dimorphism ($p = .008$, paired t -test). (d) Length of genital cortex of males versus females ($p = .99$, paired t test; error bars: SEM). (e) Same as D but the area of the cortical penis and clitoris representation is plotted against each other ($p = .052$, paired t test). (f) Ratio of penis to clitoris of external genitals is several-fold larger than that of the cortical representation ($p = .008$, paired t test)

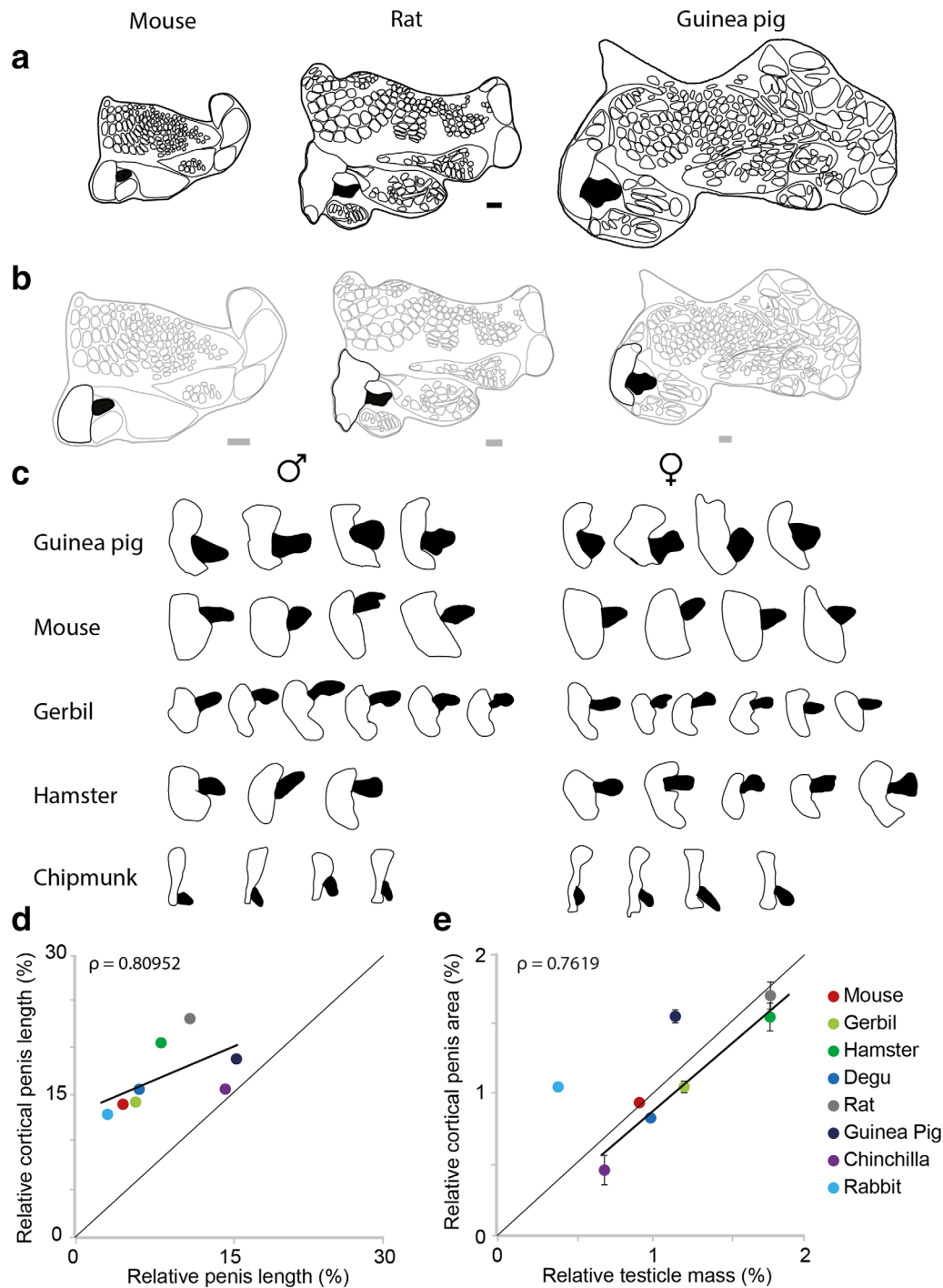


FIGURE 7 Relative size of genital cortex varies across species and correlates with markers of sexual competition. (a) Raw body maps (left: mouse; middle: rat; right: guinea pig; scale bar = 500 μm ; black: genital cortex). (b) Body maps normalized to the average size of somatosensory cortex (S1) of adult rats. Trunk and penis representation have been highlighted (scale bar = 500 μm). (c) Comparison of isolated normalized trunk and genital representation of guinea pig, mouse, gerbil, hamster, and chipmunk (left: male; right: female). All (except chipmunk) show an erect posture with a characteristic shape. Major differences were found in relative size of genital and trunk representations. (d) Relative penis length (to body length) and relative penis representation length (to head-to-tail representation length) showed high correlation ($\rho = 0.80$; $p = .021$, Spearman's rank order correlation). (e) Relative testicle mass (to body mass) and relative cortical penis area (to S1) showed high correlation ($\rho = 0.76$; $p = .036$; Spearman's rank order correlation)

discussed. Novel imaging studies (Kell, von Kriegstein, Rösler, Kleinschmidt, & Laufs, 2005) and the above mentioned recordings in monkeys (Rothmund et al., 2002) suggest a genital representation in the region of the hip and leg representation, whereas other imaging studies

(Komisaruk et al. 2011) and the initial recordings identified it at the medial wall, below the foot representation (Penfield & Rasmussen, 1950). Our findings corroborate the idea that somatotopy applies to genital cortex.

4.2 | Monomorphic representation of penis and clitoris

Our observations confirm the conclusion of Lenschow et al. (2016) that genital cortex is monomorphic between the sexes. This finding is all the more remarkable, as the species investigated here showed widely varying degrees of sexual dimorphism of external genitals. These findings suggest two conclusions: (a) the size of genital cortex is not dictated by the size of the external genital and (b) some as of yet unidentified mechanism maintains the same size of genital representation in both sexes. Previous studies of genital innervation do not resolve this issue. The pudendal nerve, which joins genital afferences, was found to have a higher number of sensory afferents in male rats compared to female rats (McKenna & Nadelhaft, 1986). A more detailed study of the dorsal penile and clitoral nerve revealed further sex differences (Moore & White, 1996). For example, the dorsal penis nerve had a significantly higher diameter and number of unmyelinated axons in comparison to the dorsal clitoral nerve. Further differences were also found, e.g., a higher degree of branching of the dorsal clitoris nerve. While sexual dimorphism can be found in some brain areas, such as the medial preoptic area (Gorski, Gordon, Shryne, & Southam, 1978), the extent to which the brain can be categorized as 'male' or 'female' is still unclear (Joel et al., 2015; Glezerman, 2016; Joel, Hänggi, & Pool, 2016). Joel et al. argue that highly sex-dependent dimorphisms are rare and propose a sex-independent mosaic brain with a diverse degree of overlaps between the sexes. Results from Lenschow et al. (2016) and our study support this view, showing that cortical genital representations are sexually monomorphic.

4.3 | Genital cortex growth during puberty

We show that the putative genital cortex protrusion greatly expands during puberty in all species studied. This observation is in line with the findings of Lenschow et al. (2016), who also reported significant differences in the relative area: the genital cortex takes up in S1 between prepubertal and adult rats. Indeed, subsequent work showed that sex hormones and sexual experience control the development of genital cortex (Lenschow and Brecht, in revision).

4.4 | Genital cortex relative size, function and sexual selection

Both the absolute areal size of genital cortex and the relative size of genital cortex in relation to the entire somatosensory cortex differed markedly between species. The relative size of genital cortex ranged from 0.5% to 1.7% of total S1 area. It has been postulated that the size of cortical sensory systems scales to the external density of receptors (Adrian, 1928; Kaas, 1997). It is most likely, that the genital cortex in S1 receives input from the dorsal penile/clitoral nerve, which is the primary sensory innervation of the external genital (Yang & Bradley, 1998). Animals, in which the nerve has been damaged, showed uncommon behaviors (primates [Herbert, 1973], rat [Larsson & Södersten, 1973]). For example, male rats still tried to mount females, but often failed at intromission or ejaculation.

The species investigated in this study show very different forms of socio-sexual behavior. Since we observed a big variation of the relative genital cortex area (0.5–1.7% of S1), we wanted to know whether there is a linkage between behavior and relative cortical representation. Rats, mice, guinea pigs, hamsters, rabbits, degus, and chipmunks show polygamous behavior (Nowak, 1999; Brower, 2006; Rieger, 2014); gerbils and chinchilla show monogamous pair formation (Ågren, 1984; Norris & Adams, 1972; Brower, 2006). Hamsters and chipmunks live solitarily (Elliot, 1978; Wolff, 2007; Siegel, 1985); the other species either live in groups, pairs or harems (Nowak, 1999; Brower, 2006; Rieger, 2014). Furthermore, the species show different mating behaviors. Hamsters and rats mate with short intromissions in a high frequency, mice show a small number of intromissions until ejaculation and guinea pigs and chinchillas ejaculate frequently while intromitting (Bignami & Beach, 1968). Due to the small number of different species investigated, a strict categorization either did not show significant effects or could not be analyzed in a statistically meaningful way. Additionally, a categorization is problematic, because e.g., real monogamy is rare (Wolff, 2007) or animals show deviant sexual behavior, if bred in the laboratory (Brower, 2006; Norris & Adams, 1972). Thus, given our small sample size we could not establish a link between mating systems and genital cortex size.

Another type of analysis, which focused on the relationship between genital cortex proportions and sexual selection, proved to be more fruitful, however. We used markers for sperm competition and sexual selection, such as relative testicular size or relative penis length. Species with a higher degree of sperm competition show a higher testicular size (Parker, Ball, Stockley, & Gage, 1997; Birkhead & Møller, 1998; Gage & Freckleton, 2003). By increasing the testicular size, production of sperm is higher (Parker & Ball, 2005), enabling a higher amount of ejaculations per hour (Birkhead & Møller, 1998) and resulting in greater reproductive success (Parker & Ball, 2005). Furthermore, Ramm (2007) has shown that the ratio of the glans penis and baculum length to body length correlate with relative testicular size and sperm competition in rodents. Our data revealed a significant correlation of relative genital cortex morphology with sperm competition markers. The fraction of genital cortex in S1 is correlated with relative testicular size. Additionally, the relative length of genital cortex correlated significantly with the relative length of the external genital. We conclude that sexual selection is a driving force in the evolution of genital cortex. To our knowledge, this is the first time that sexual selection and evolution of the cortex can be linked directly.

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CONFLICT OF INTEREST

All authors declare they have no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: SML, CL, MB. Acquisition of data: SML. Analysis and interpretation of data: SML, CL, MB. Drafting of the manuscript: SML, MB. Statistical analysis: SML. Obtained funding: MB. Administrative, technical, and material support: MB. Study supervision: MB.

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