Sexually Monomorphic Maps and Dimorphic Responses in Rat Genital Cortex

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Highlights:

1. We identified maps of genitals in rat somatosensory cortex

2. Cortical genital maps are sexually monomorphic despite genital dimorphism

3. Responses of genital neurons were sexually dimorphic

eTOC

Lenschow et al. used physiological and anatomical mapping techniques to uncover a large and robust genital representation in a region of rat somatosensory cortex previously assigned as leg/forelimb cortex. Despite the marked sexual dimorphism of rat external genitals, anatomical cortical maps of penis and clitoris showed a stunning monomorphism.

Summary

Mammalian external genitals show sexual dimorphism [1,2] and can change size and shape upon sexual arousal. Genitals feature prominently in the oldest pieces of figural art [3] and phallic depictions of penises informed psychoanalytic thought about sexuality [4, 5]. Despite this longstanding interest, the neural representations of genitals are still poorly understood [6]. In somatosensory cortex specifically, many studies did not detect any cortical representation of genitals [7-9]. Studies in humans debate, if genitals are represented displaced below the foot of the cortical body map [10-12], or if they are represented somatotopically [13-15]. We wondered, what a high-resolution mapping of genital representations might tell us about the sexual differentiation of the mammalian brain. We identified genital responses in rat somatosensory cortex in a region previously assigned as arm/leg cortex. Genital responses were more common in males than in females. Despite such response dimorphism, we observed a stunning anatomical monomorphism of cortical penis and clitoris input maps revealed by cytochrome-oxidase-staining of cortical layer-4. Genital representations were somatotopic, bilaterally symmetric and their relative size increased markedly during puberty. Size, shape and erect posture give the cortical penis representation a phallic appearance pointing to a role in sexually aroused states. Cortical genital neurons showed unusual multi-body-part responses and sexually dimorphic receptive fields. Specifically, genital neurons were co-activated by distant body regions, which are touched during mounting in the respective sex. Genital maps indicate a deep homology of penis and clitoris representations in line with a fundamentally bi-sexual layout [16] of the vertebrate brain.

Results

Rat external genitals were sexually dimorphic. Scrotum and vulva were only present in males (Figure 1A) and females (Figure 1B), respectively. The penis and external clitoris had a similar shape but show a several-fold size difference (right panels in Figure 1A and 1B).

For high-resolution mapping of genital cortex we combined physiological and anatomical histochemistry-based analysis. Anatomical cytochrome-oxidase maps are much clearer in young animals [17]. Hence, we focused our initial experiments on young prepubescent animals. For physiological mapping of posterior rat somatosensory cortex, we assessed tactile receptive fields of multi-unit and in rare cases also single-unit responses at 377 penetration sites in 6 male and 5 female prepubescent rats. To plot receptive fields a recording pipette was inserted at a depth ranging from deep cortical layer 3 to upper layer 5. For neural recordings signals were filtered for spikes and sent to an audio-monitor, while palpating the animal's body surface. These procedures typically resulted in multi-unit recordings, but in a small fraction of cases (~10%) we also encountered isolated single-unit responses.

A male map is shown in Figure 1C. Genital responses were observed in an area around 2.5 mm lateral and 2.5 mm posterior from bregma. A similar location of genital responses was seen in a female animal (Figure 1D). Pure genital responses (black) were rare and in most instances genital responses overlapped with responses to different body parts (striped in Figure 1C and 1D).

When averaging individual response maps by sex, we found the strongest genital responses in males for palpation of the scrotum at 2.5 mm posterior and 2 mm lateral from bregma (Figure S1A). In females, we found the strongest response 2.5 mm posterior and 2.5 mm lateral from bregma, corresponding to the vulva (Figure S1B). Interestingly, in males, we detected another site of strong genital responses at 1.5 mm posterior and 3.5 lateral from bregma, where we mainly observed responses to penis palpation (Figure S1A). The clitoris had no well-defined best average site of responsiveness. In individual experiments, however, clitoris responses were observed anterior and lateral from vulva responsive sites. As already noted the genitals were rarely the sole responsive area. In particular, there was often overlap with the cortical regions receptive to the forelimb and the trunk as coarsely outlined in the schematics of male and female somatotopy (Figure 1E and 1F). We found that males show a significantly higher fraction of genital responses ~28% (55 out of 194 sites) than females, which showed genital responses only at ~16% (30 out of 183 sites, $P = 0.007$, Fisher's exact test).

After receptive field mapping we obtained detailed anatomical maps of layer 4 in tangential sections through the somatosensory cortex. To this end cytochrome oxidase staining was performed, which reveals granular layer 4 regions (by a dark precipitate, Figure 2A and 2B). Granular zones of sensory cortex are characterized by numerous layer 4 granule cells and receive massive thalamic inputs. These histochemically delineated maps were vivid and much more detailed than any physiological mapping result. We identified the genital representation in these anatomical maps by three approaches: (i) We placed lesions at sites responsive to genitals (n = 2, one penis response lesion, one clitoris response lesion). (ii) We matched individual physiological maps to the overall layout of individual anatomical maps and then asked which part of the anatomical map corresponds to the genital (n = 10). To this end, we identified 4 landmarks (hindpaw, forepaw, trunk and unresponsive zone posterior to the trunk), which could be easily identified in physiological maps and anatomical maps. Once we achieved an optimal alignment of these landmarks, we asked which part of the anatomical map corresponded to the genital responses. (iii) For hemispheres (n = 17), where we did not obtain physiological mapping data, we used the same procedure to match anatomical maps to our overall physiological maps (Figure 1E and 1F) and published response maps [7-9]. All three methods led to the same conclusions, as depicted in Figure 2C and 2D. The anatomical maps of male (Figure 2A and 2C) and female (Figure 2B and 2D) genitals allowed two striking observations. First, cortical male penis and female clitoris were very similar, i.e. genital maps were sexually monomorphic. Second, the anatomical penis map looked different from unlike the flaccid (resting) penis (Figure 1A left), which was small, pointing downward and aligned to the scrotum. Instead, size, shape and erect posture give the cortical penis map a phallic appearance. We noted a substantial individual variability of cortical genital representations, but found them to be bilaterally symmetric both in males (Figure 2E) and females (Figure 2F). We measured the area of various somatosensory areas by outlining the anatomical regions of interest (n = 11 male, 6 female hemispheres). These measurements confirmed the quantitative similarity of cortical penis, and clitoris representation with respect to area (Figure 2G), and cortical genital length (Figure 2F). Our detailed measurements included shaft length (from the tip of the genital representation to its base), width (width half way from the base), and the length from the tip to the back of the trunk (total genital length, which includes scrotum and vulva representations respectively). All measurements confirmed sexual monomorphism.

As previous mapping studies did not report genital responses in rat somatosensory cortex, we wanted to confirm the presence of such responses by more objective method than the mere hand mapping of receptive fields. To this end, we obtained *in vivo* whole-cell recordings (N = 10) at

the coordinates identified as genital cortex by mapping experiments and applied air-puff stimuli to the genitals. As shown in Figure S2 we observed huge (up to 25 mV average) postsynaptic responses to genital stimulation in such recordings. Within the genital region we often observed marked response differences (compare the scrotum response in Figure S2A to the penis response Figure S2B) indicating relatively small receptive fields within the genital region. Taken together the analysis of postsynaptic responses corroborated the presence of a genital representation in rat somatosensory cortex.

While anatomical genital maps were surprisingly sexually monomorphic, close inspection of cortical genital receptive fields revealed sex differences. Both male (Figure 3A) and female (Figure 3B) genital responses were rarely observed in isolation and co-localized with other body parts (Figure 3A-C). We mapped 55 genital receptive fields in the males and found that 13 of these receptive fields included genitals and forearm/forepaw/shoulder. In females we identified 30 genital receptive fields and only 2 fields co-localized with the forearm. Thus, the fraction of forelimb/genital receptive fields is significantly smaller in females (Fisher's exact test, Figure 3C). In females, many genital receptive fields showed co-localization with the trunk (15 out of 30), whereas such co-localization was rare in males (7 out of 55). This difference was significant (Figure 3A-3C, Fisher's exact test). We were concerned that the unusual multi-body-part response could be an artifact of the mixing of single-body-part responses of different cells in our multi-unit recordings. Therefore, we performed single-unit recordings in genital cortex (N=24 cells in males and N=24 cells in females). In both sexes a majority of cells increased their firing rate after genital air puffs, but many single units responded sparsely. We found that single genital neurons responded to multiple body parts (Figure 3D upper). In pooled responses of those cells that showed at least some ($\geq$ 0.2 Hz) ongoing activity (Figure 3D lower) forearm-genital combinations were more common in males than in females (Figure 3E upper and lower), as observed before for multi-unit responses. On the other hand, trunk-genital combinations were seen more often in female single neuron responses (data not shown).

The data presented so far referred to young prepubescent animals. As genitals do not acquire their full functionality until adulthood, we wondered, if genital maps change in older animals. As expected [17], adult cytochrome oxidase maps were less clearly delineated (Figure S3A and S3B), but had qualitatively the same layout as in young animals (Figure S3C and S3D). Most interestingly, however, we observed a massive size increase of genital cortex during puberty (Figure 4A). While the overall size of somatosensory cortex increased only modestly (+14%) between ~P25 animals and animals P42 and older (Figure 4A, B), the clitoris and the penis representation both roughly doubled in size (Figure 4A, C). The hind-paw representation also

increased in relative size (Figure 4D), whereas the forelimb representation did not change much in puberty (Figure 4E). To assess relative growth we normalized for each hemisphere and each body part the size of adult representations by dividing it by the average size of this body part in maps from young animals. We found that there was a significant difference in relative growth between body parts (Anova, $P < 0.05$). We then used unpaired t-Tests to compare the growth of genital cortex (penis and clitoris representation) to growth of other body parts. We found that genital cortex grows significantly more than the entire S1, the trunk, and the forepaw (two-tailed t-Test, $P < 0.05$). In contrast, genital cortex did not grow significantly more than hindpaw cortex (two-tailed t-Test, $P = 0.06$). The size increase of the genital representation during puberty was the same in both sexes. Thus, the mean relative size of clitoris and penis representation was almost exactly the same between adults of both sexes (1.73% vs. 1.71 % of somatosensory cortex in males vs. females). Given the marked expansion of the cortical genital representation in puberty we wondered how genitals change in puberty. In line with previous studies [18] we observed a substantial length increase in the penis (almost a doubling of penis length), but only a minor (10-20%) length increase of the clitoris in puberty. Thus, the cortical monomorphism of genital representation persists through puberty despite increasing external sexual dimorphism.

Discussion

Physiological and anatomical mapping techniques revealed a large and robust genital representation in a region of rat somatosensory cortex previously assigned as leg/forelimb cortex [7-9]. This discrepancy with earlier work might stem from: (i) a lack of focus on genitals previously, (ii) the fact that genitals are poorly accessible for mapping (because of their very posterior, ventral and partially internal position), (iii) the unusual overlap of genital receptive fields with other body parts, which can obscure genital responses, (iv) the use of adult animals in previous mapping work, which complicates cytochrome-oxidase based anatomy.

We detected a number of sex differences in genital cortex. The young, prepubescent male rats studied here showed a larger fraction of genital responses than prepubescent female rats. In light of the pronounced growth of genital cortex during puberty it would be worthwhile to reinvestigate cortical responses in adult animals and in different sexual states (i.e. estrus vs. nonestrus). Earlier work in cats and rats indicated a modulation of genital sensory responses by estrus [19, 20] and other findings suggest a differential processing of sexual information through stages of the estrus cycle. Different from males, females show cyclic peaks in sexual desire and excitability around the time of ovulation [21]. Modulation of cortical representation by maternity has been documented in somatosensory cortex [22] and auditory cortex [23-25]. Whether genital cortex responses are cycling with the sexual state of females, as it was shown in the ventromedial hypothalamus of mice [26], needs to be explored in recordings in awake animals.

Receptive field structure was sexually dimorphic in the genital cortex. In males genital responses often combined with forelimb responses, while in females we found genital and trunk composite receptive fields. Such multi-body-part receptive fields are quite rare in other parts of somatosensory cortex. Male forelimb/genital fields might be explained by the extension of the cortical penis representation to the forelimb in the body map, but this explanation cannot account for the absence of such fields in females. Such sexually dimorphic receptive fields might reflect a sexual function: the body parts co-represented with genitalia are those parts contacted in males and females during mounting.

We discovered monomorphic anatomical maps of penis and clitoris in layer 4 of the somatosensory cortex. Mapping by cytochrome oxidase histochemistry offers a much higher (~ 5 μm vs. several 100 μm) resolution than previous physiological maps. The vivid body and genital maps provide clear evidence for a somatotopic genital representation [13-15]. Anatomical cortical genital maps are remarkable for five reasons: First, such high-resolution

maps allow precise delineation of cortical genital maps rather than a mere 'symbolic' genital illustration [11, 14]. Second, size, shape and erect posture give the cortical penis representation a phallic appearance pointing to a role of genital cortex in sexually aroused states. Hence, illustration of genital sensations by a non-erect penis in Cantlie/Penfield & Rasmussen [11] and Kell et al. [14] is likely to be incorrect. We also visualized a phallic clitoris representation, which is interesting in the context of lesbian female phallus proposals [27]. Third, the growth of genital cortex in puberty is a highly unusual developmental pattern, because (i) of its magnitude, which exceeds the growth of somatosensory cortex in the entire postnatal life [28], (ii) it alters layer 4 input maps, which usually become immutable shortly after birth [29,30] (iii) it occurs so late in postnatal development. Such findings point to potential neural substrates of the marked changes in sexual behavior during puberty and indicate that there is not a single critical period for the entire somatosensory cortex. Fourth, the identification of cortical genital maps opens up new avenues for the study of sexuality, much like the discovery of an anatomical barrel map [31] inspired studies on the whisker system [32, 33]. Similarly, cortical genital maps should be instrumental in delineating cortical sexual information flow, which can now be approached by determining the connectivity of genital cortex. Bilaterally-symmetric genital maps contrast with evidence from stroke patients for right hemispheric sexual lateralization [34], a discrepancy that deserves further attention. Fifth, these maps reveal a cortical monomorphism of penis and clitoris representation. Such monomorphism is entirely unexpected in light of the marked external genital dimorphism. In line with genital dimorphism some authors observed an innervation of male genitals by more afferents [35] or at least by larger afferents [36] than in females. This puzzling monomorphism might be related to the common developmental origin of penis and clitoris from the genital tubercle.

Conclusion

Combined physiological and anatomical mapping of genital representations results in high-resolution cortical genital maps. Such maps are much different from previous work, which identified the putative cortical location of genital sensations, but represented cortical genitals only symbolically [10, 11, 14]. The striking map monomorphism of cortical genital representations might be best understood in the context of developmental [37], neurogenetic [38] and comparative work argued against a purely genetic sexual determination of the vertebrate brain. In particular, neuro-endocrinological analysis of pseudo-sexual parthenogenetic lizards suggested that sex hormones impose sexual identity on a sexually

plastic brain with bi-sexual potential [16]. Thus, we argue that monomorphic genital maps reflect the fundamentally bi-sexual layout of the vertebrate brain.

Experimental Procedures

For details, please see the Supplemental Experimental Procedures. All experimental procedures were performed according to German and American regulations on animal welfare and were approved by ethics committees in Berlin, Germany, and Woods Hole, MA, respectively. Long-Evans rats were provided by the Marine Biological Institute, Woods Hole (USA). Prepubescent animals for histological and physiological mapping were aged between post-natal day (P) 23 and P30. Adult animals for histological analysis were between 6 weeks and one year old. Long-Evans rats (P22–P30, n = 11, 6 males and 5 females) were anesthetized using urethane (1.4 g/kg, i.p.). An approximately 5 x 5 mm sized craniotomy was made 5 mm posterior to and 5 mm lateral to bregma. At each recording site we searched for clear tactile responses at a depth between deeper layer 3 (600 μm) to upper layer 5 (1300 μm) and plotted receptive fields. Receptive fields plotted by systematically palpating the animal's body surface including the internal parts of the vulva/clitoris in females. For single-unit recordings we used 5 Mega Ohm glass pipettes and recorded large (> 0.5 mV) spikes of individual cells in the juxtacellular configuration. After physiological mapping, animals received an overdose of the anesthetic (20% urethane solution) and were perfused with phosphate buffer followed by a 4% paraformaldehyde solution (PFA). Brains were removed, hemispheres were separated, and cortices were flattened between two glass slides separated by clay spacers. Sections were stained for cytochromeoxidase activity using the protocol of Wong-Riley [39]. Subsequently, granular somatosensory regions (indicated by a dark precipitate from the cytochrome oxidase stain) were drawn with ImageJ software. The area of various somatosensory regions was outlined and measured by the ImageJ area calculating tool (see Fig. 2C). The anatomical maps were matched to the physiological mapping by: (i) individual recording sites were matched to anatomical map locations by placing electrolytic lesions. Lesions were placed by injecting 10 μA negative current through a tungsten electrode for 10 s. (ii), individual physiological maps were matched to the overall layout of individual anatomical maps (n = 10). (iii) anatomical maps (n = 17 hemispheres/maps from young animals and n = 9 hemispheres/maps from adult animals) were matched to our overall maps (Figure 1E and 1F) and published maps [7-9].

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Contributions C.L., S.C., J.M.G., Z.N.T. and A.V. performed experiments and analysis. M.B. supervised the study. All authors contributed to writing the manuscript.

Figure Legends

Figure 1: Physiological mapping of cortical genital responses in male and female rats

(A) Male rat genitals in low (left) and high (right) magnification views.

(B) Female rat external genitals as in A. Note the marked size dimorphism of penis and clitoris.

(C) Left, a physiological map of male posterior somatosensory cortex. Mapping sites are indicated relative to bregma. Colors indicate responses to different body parts. Depending on the spacing of mapping penetrations, responses were depicted by squares referring to 0.5 x 0.5 mm or by rectangles referring 0.5 x 0.25 mm of cortical area. Right, schematic view of the rat brain with the area of cortical mapping indicated and legend. The lesion was placed (red star) at a site, which responded strongly to penis and weakly to scrotum palpation. Sites responsive to penis and scrotum are referred to as genital sites.

(D) Same as **C**, for a female map. Sites responsive to clitoris and vulva are referred to as genital sites.

(E) Averaged positions of all mapped genital (grey transparent) and other body part responses assembled to a schematic overview map for males.

(F) Same as **E**, but for females.

See also Figure S1.

Figure 2: Anatomical maps of genitals in somatosensory cortex revealed by cytochrome oxidase stains

(A) Cytochrome oxidase staining of a tangential section through layer 4 of male somatosensory cortex. The dark precipitate reveals granular parts of layer 4, with numerous granule cells and massive thalamic inputs. A = anterior, L = lateral.

(B) Cytochrome oxidase map of female somatosensory cortex, conventions as in **A.**

(C) Outline of male 'ratunculus'. Green lines and arrows show how measurements were taken for various lengths of the genital representation including width at half-length, total genital length, and shaft length. Note that neurons in region labeled as penis did not exclusively respond to penis stimulation, but that a fraction of neurons in this region also responded to other body parts.

(D) Drawing of female 'ratunculus', conventions as in **C**. Note that neurons in region labeled as clitoris did not exclusively respond to clitoris stimulation, but that a fraction of neurons in this region also responded to other body parts.

(E) Examples of male granular cortex body (gray) and penis (black) representations in left (LH) and right (RH) hemispheres of two male rats. Outlines were drawn for one section, which best (and completely) represented the genitals. Body and trunk may be partially incomplete. The red star marks the position of lesion placed on a site responsive to cutaneous stimulation of the penis; see Fig. 1C.

(F) Drawing of female cortical body (gray) and clitoris (black) representations, conventions as in **E**. Note the bilateral symmetry of genital representations in **E** and **F**.

(G) Area of various cortical somatosensory regions, for both female (red) and male (blue) rats; areas measured include the clitoris, penis, trunk, forearm, and hind-paw.

(H) Lengths of cortical genital regions, measurements as indicated by the green lines in **C**. Length and areal measurements refer to n = 11 male, 6 female hemispheres.

Error bars depict standard errors of the mean. See also Figure S2.

Figure 3: Sexually dimorphic multi-body-part receptive fields in genital somatosensory cortex

(A) Co-localization of male genital receptive fields with forearm and anterior trunk. Ten genital receptive fields from ten recording sites in one experiment on a male rat are drawn on a ventral view (upper) and a side view (lower) of a rat. Most receptive fields co-represent genitals with forearm and anterior trunk.

(B) Co-localization of female genital receptive fields with posterior trunk, hind limb and tail. Seven genital receptive fields from seven recording sites in one experiment on a female rat are drawn on a ventral view (upper) and a side view (lower) of a rat. Most receptive fields co-represent genitals with the hindlimb and posterior trunk.

(C) Quantification of male and female genital receptive fields patterns (blue and red respectively) across experiments. In males genital responses co-localized significantly more with the forelimb than in females. In females genital responses co-localized significantly more often with the trunk than in males. Pure genital receptive fields were rare. We compared the occurrence of receptive field combinations in males and females with Fisher's exact test. Not all receptive field combinations encountered are listed. Note that the receptive field locations match the expected physical contact patterns when the male animal (A lower) mounts the female (B lower).

(D) Upper panel: PSTH of activity of a single neuron recorded from male genital cortex aligned to the onset of a penis air puff (left) and a forearm air puff (right). Note the cell's responsiveness to both penis and forearm stimulation. Lower panel: Pooled PSTH of activity of 10 cells recorded in male genital cortex aligned to the onset of penis air-puff stimulation (left) and forearm air-puff stimulation. PSTHs were normalized such that each cell contributed equally; only cells with ongoing firing rates > 0.2 Hz were included.

(E) Upper panel: Same as D but PSTH is shown for a neuron recorded in female genital cortex. Compared to the PSTHs in males, the example cell shows no responses to forearm stimulation. Lower panel: Pooled PSTH of activity of 8 cells recorded from female genital cortex. Clitoris responses were pronounced whereas forearm stimulation in the same cells shows no responses. Conventions as in **D**.

Figure 4: Adult genital maps and growth of genital cortex during puberty

(A) Upper panel, drawing of a complete somatosenory cortex (thick outline) body map of a prepubescent female (age: P25). The drawing was compiled by tracing barrels/body-parts (thin outlines) through several tangential cortical sections stained for cytochrome-oxidase activity. Black, clitoris area. Middle, average size increase of the representation of different body parts (S1 = entire somatosensory cortex, PMBSF = Postero-Medial-Barrel-Sub-Field) in somatosensory cortex from prepubescent to post-pubescent females (N=5 and N=4, respectively). Lower panel, drawing of a complete somatosensory cortex body map of post-pubescent female (age: P42). Note that in the younger animal more body-part substructure (barrels) could be resolved than in the older animal.

(B) Absolute area of somatosensory cortex (S1) in hemispheres of prepubescent (N=5 males and N=5 females) and of post-pubescent animals (N=5 males and N=4 females).

(C) Fraction of penis (blue) and clitoris (red) cortex of the entire somatosensory cortex (S1) in hemispheres of prepubescent (N=5 males and N=5 females) and of post-pubescent animals (N=5 males and N=4 females).

(D) Fraction of hind-paw cortex of the entire somatosensory cortex (S1) in hemispheres of prepubescent (N=5 males and N=5 females) and of post-pubescent animals (N=5 males and N=4 females).

(E) Fraction of forepaw cortex. Conventions as in **D**.

Area sizes in prepubescent and post-pubescent animals were compared by unpaired two-tailed t-Tests.

See also Figure S3.

Figure 1

Click here to download Figure Fig-1 Lenschow.tif

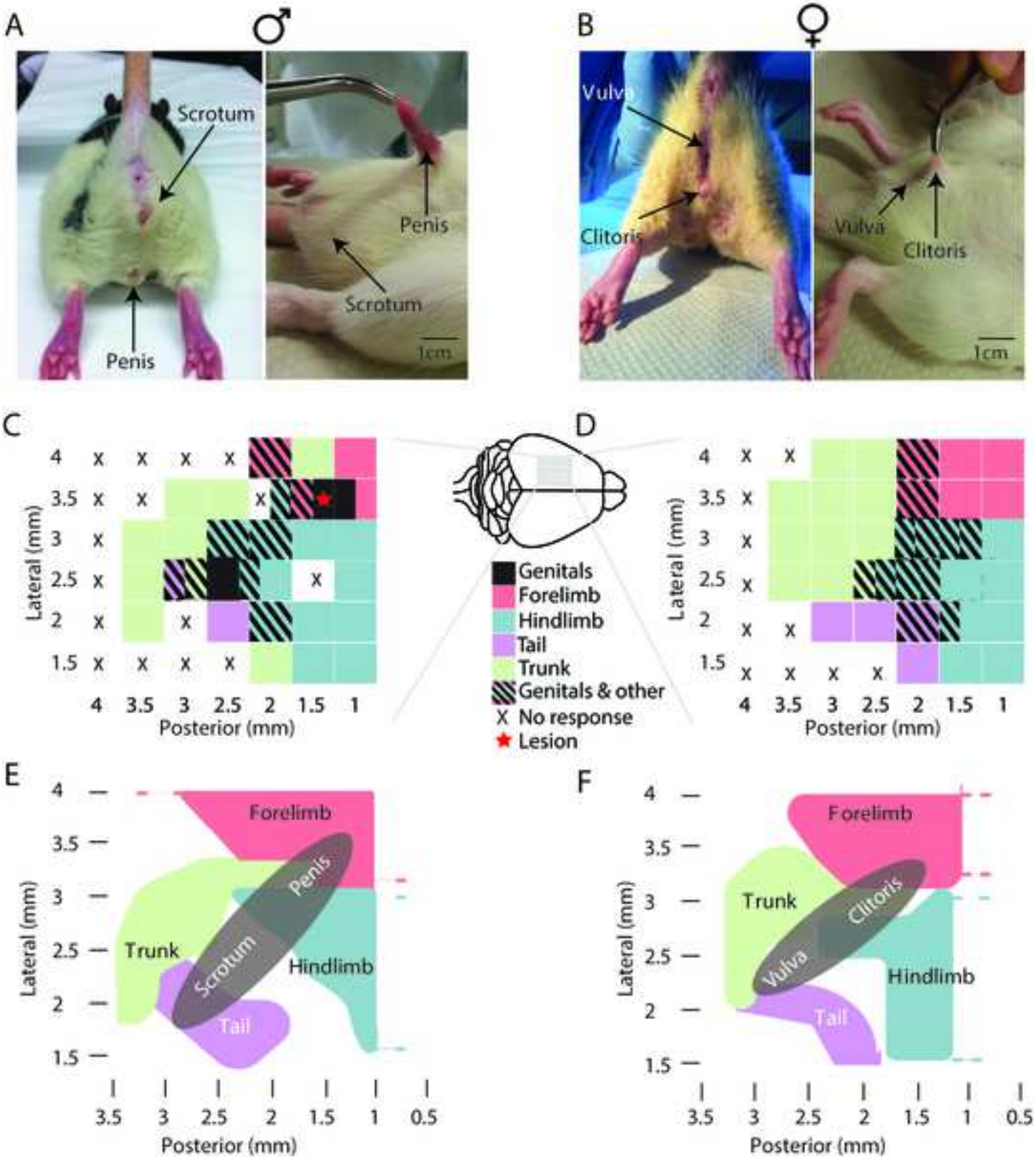


Figure 2

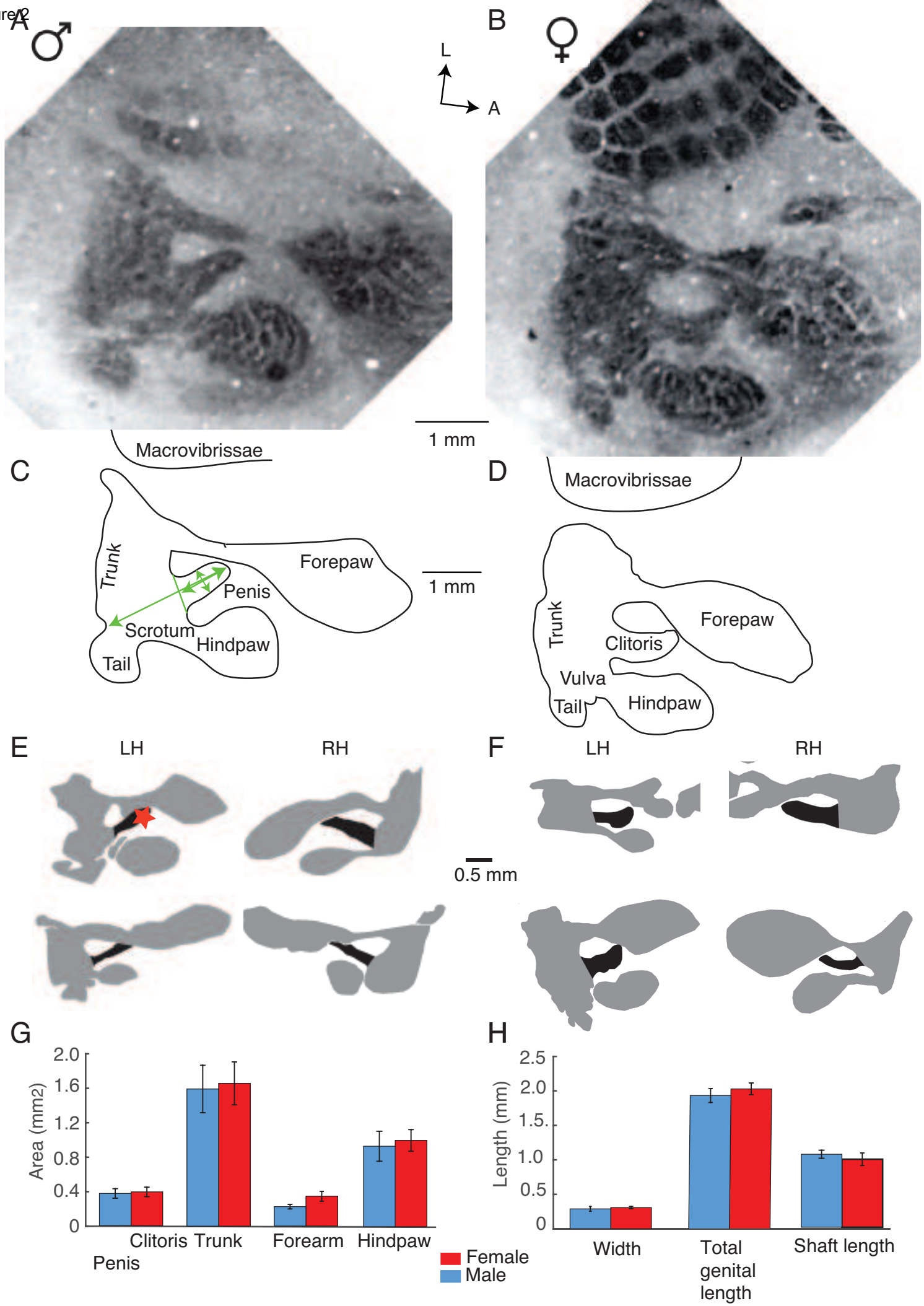

A
B
L
A
1 mm
C
Macrovibrissae
Trunk
Forepaw
Penis
Scrotum
Hindpaw
Tail
1 mm
D
Macrovibrissae
Trunk
Forepaw
Clitoris
Vulva
Tail
Hindpaw
E
LH
RH
F
LH
RH
0.5 mm
G
Area (mm2)
2.0
1.6
1.2
0.8
0.4
0
Clitoris
Penis
Trunk
Forearm
Hindpaw
Female
Male
H
Length (mm)
2.5
2.0
1.5
1.0
0.5
0
Width
Total genital length
Shaft length

Figure 3

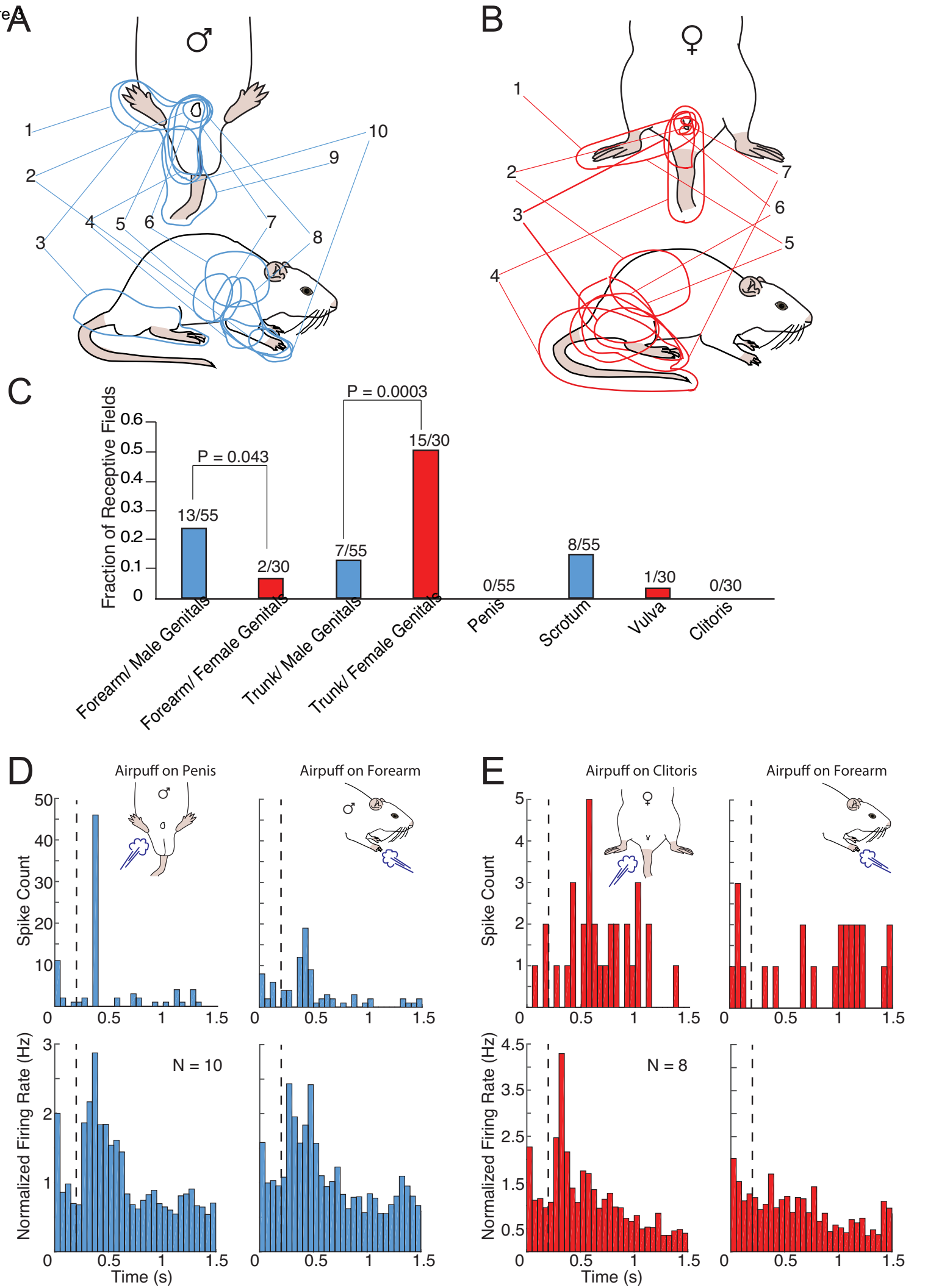
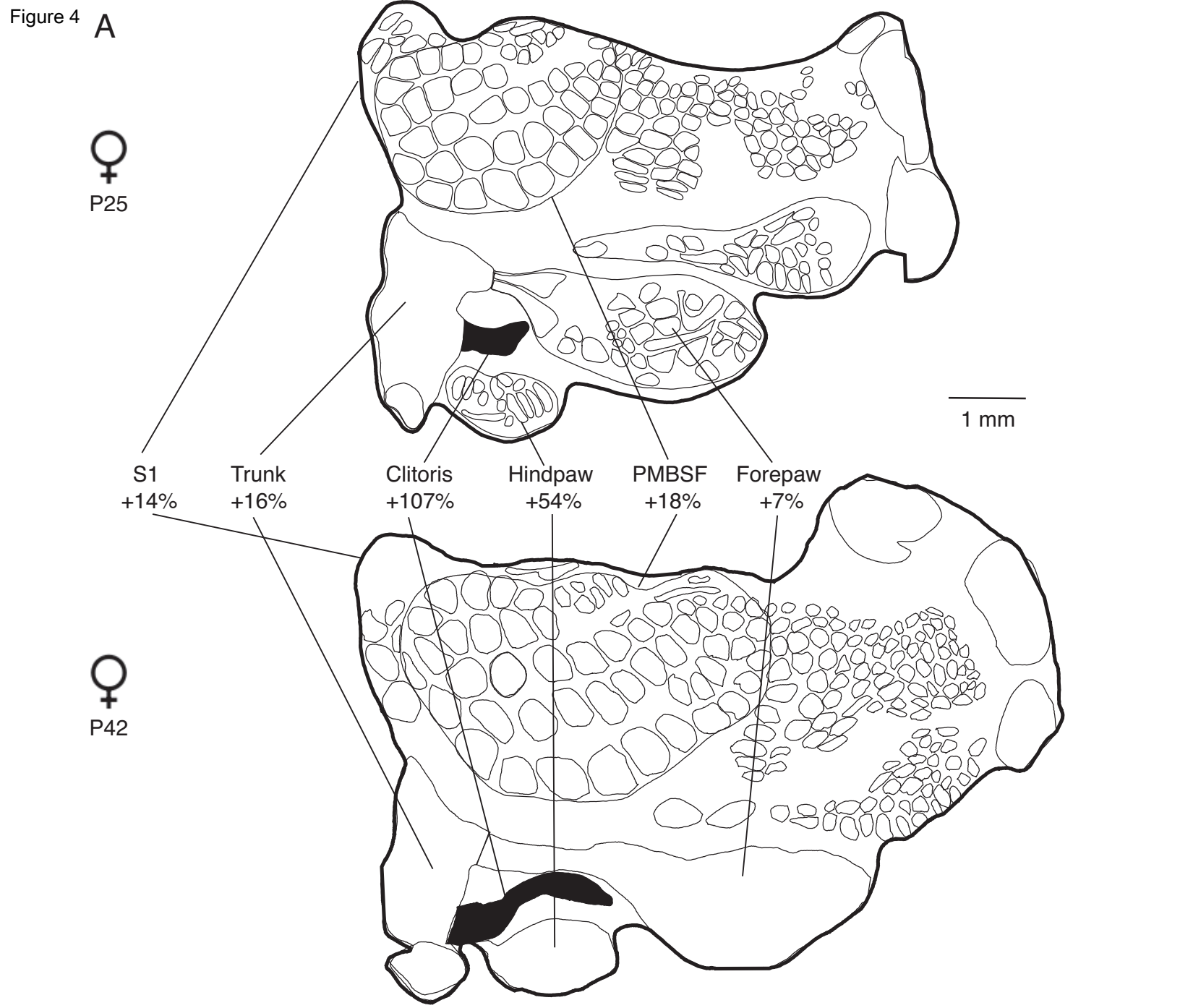
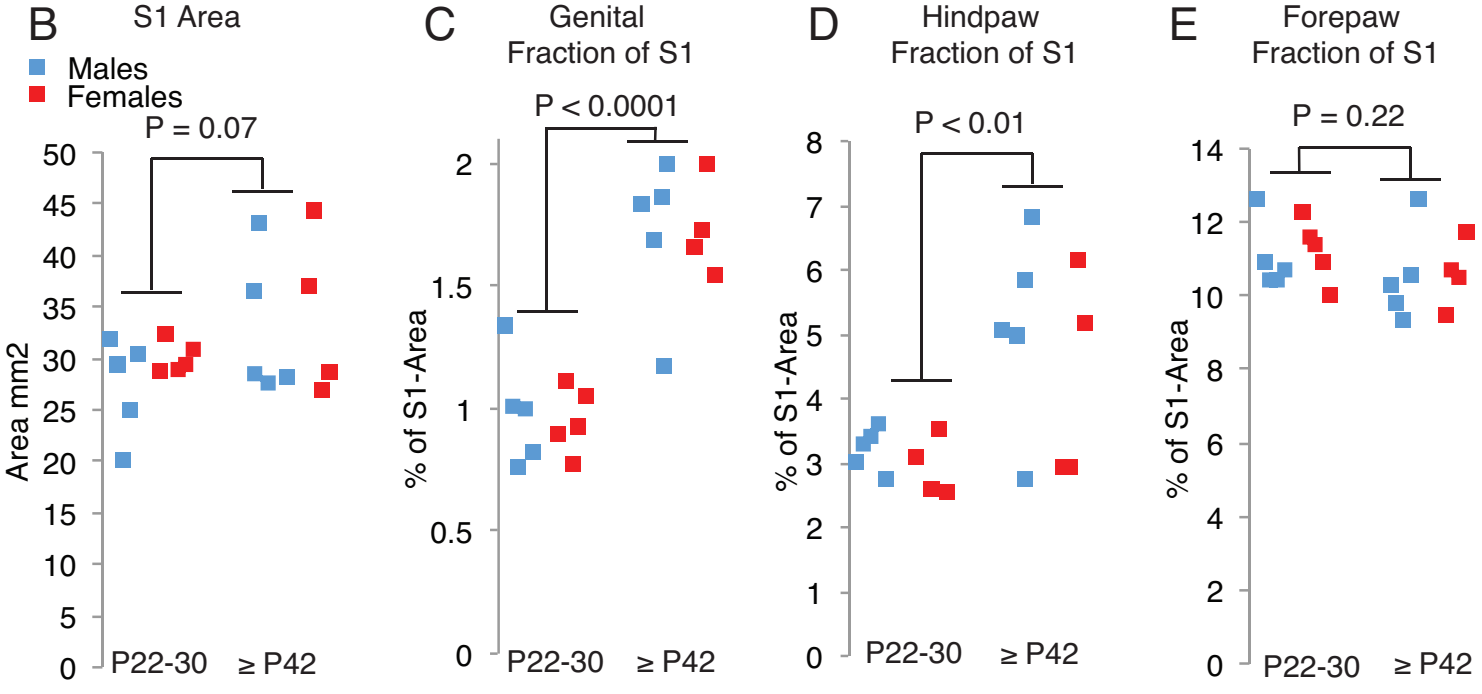

A
B
C
D
E
Fraction of Receptive Fields
P = 0.043
P = 0.0003
13/55
2/30
7/55
15/30
0/55
8/55
1/30
0/30
Forearm/ Male Genitals
Forearm/ Female Genitals
Trunk/ Male Genitals
Trunk/ Female Genitals
Penis
Scrotum
Vulva
Clitoris
Airpuff on Penis
Airpuff on Forearm
Airpuff on Clitoris
Airpuff on Forearm
Spike Count
Normalized Firing Rate (Hz)
N = 10
N = 8
Time (s)

Figure 4


A
P25
1 mm
S1
+14%
Trunk
+16%
Clitoris
+107%
Hindpaw
+54%
PMBSF
+18%
Forepaw
+7%
P42


B
S1 Area
Males
Females
P = 0.07
Area mm2
P22-30
≥ P42
C
Genital
Fraction of S1
P < 0.0001
% of S1-Area
D
Hindpaw
Fraction of S1
P < 0.01
E
Forepaw
Fraction of S1
P = 0.22

SUPPLEMENTAL INFORMATION

Sexually Monomorphic Maps and Dimorphic Responses in Rat Genital Cortex

Constanze Lenschow, Sean Copley, Jayne M. Gardiner, Zoe N. Talbot, Ariel Vitenzon and Michael Brecht

Inventory list of supplemental data

- **Supplemental Figures:**

Title:	**Related to:**
Figure S1: Averaged maps of genital responses in males and females	Figure 1
Figure S2: *In vivo* whole-cell recordings in genital cortex	Figure 2
Figure S3: Cytochrome oxidase maps of genitals in somatosensory cortex of adult animal	Figure 4

- **Supplemental Experimental Procedures**

- **Supplemental References**

SUPPLEMENTAL FIGURES

Figure S1. Related to Figure 1.

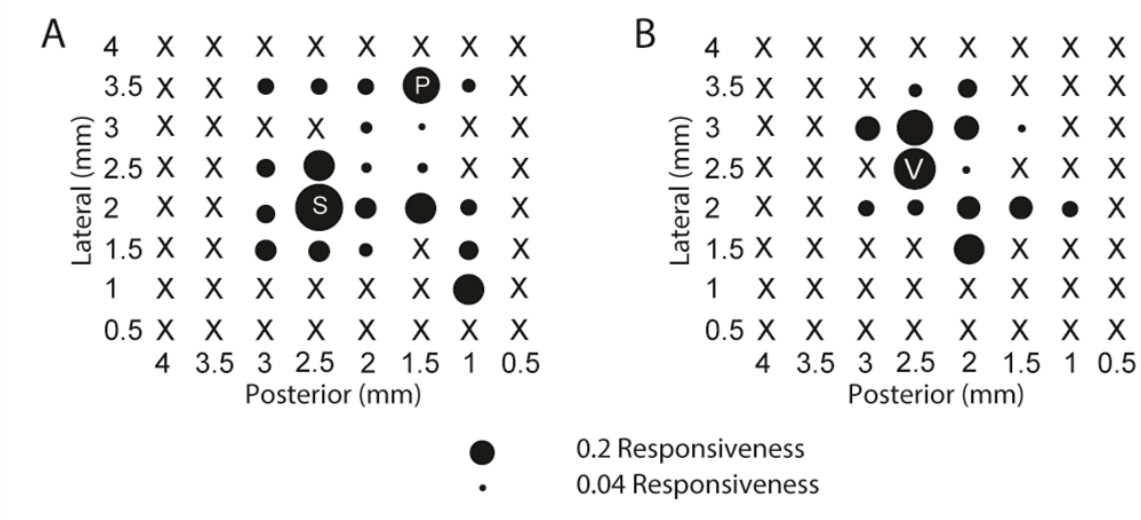


Figure S1: Averaged maps of genital responses in males and females

(A) Averaged genital responses. For each position, averaged responses to male genitals are plotted; data refer to 6 mapping experiments. In each experiment a site was scored with: 1 if it responded exclusively to genitals; 0.5 if the genitals were half of the receptive field area or the genitals included the receptive field center; 0.25 if the receptive field included the genitals but to a lesser extent; 0 if there was no genital response. An X denotes positions with response average of 0, i.e. positions, where no genital response was observed in all experiments. The diameter of the black dot linearly scales with mean average genital response score; two diameters and the responsiveness are shown below the panel. Coordinates refer to bregma. P, S average site of best penis and scrotum responsiveness, respectively.

(B) Same as **A**, but for female genital maps (average of 5 experiments). V = average site of best vulva responsiveness. The clitoris had no well-defined best site of average responsiveness. In individual experiments, however, clitoris responses were observed anterior and lateral from vulva responsive sites.

Figure S2. Related to Figure 2.

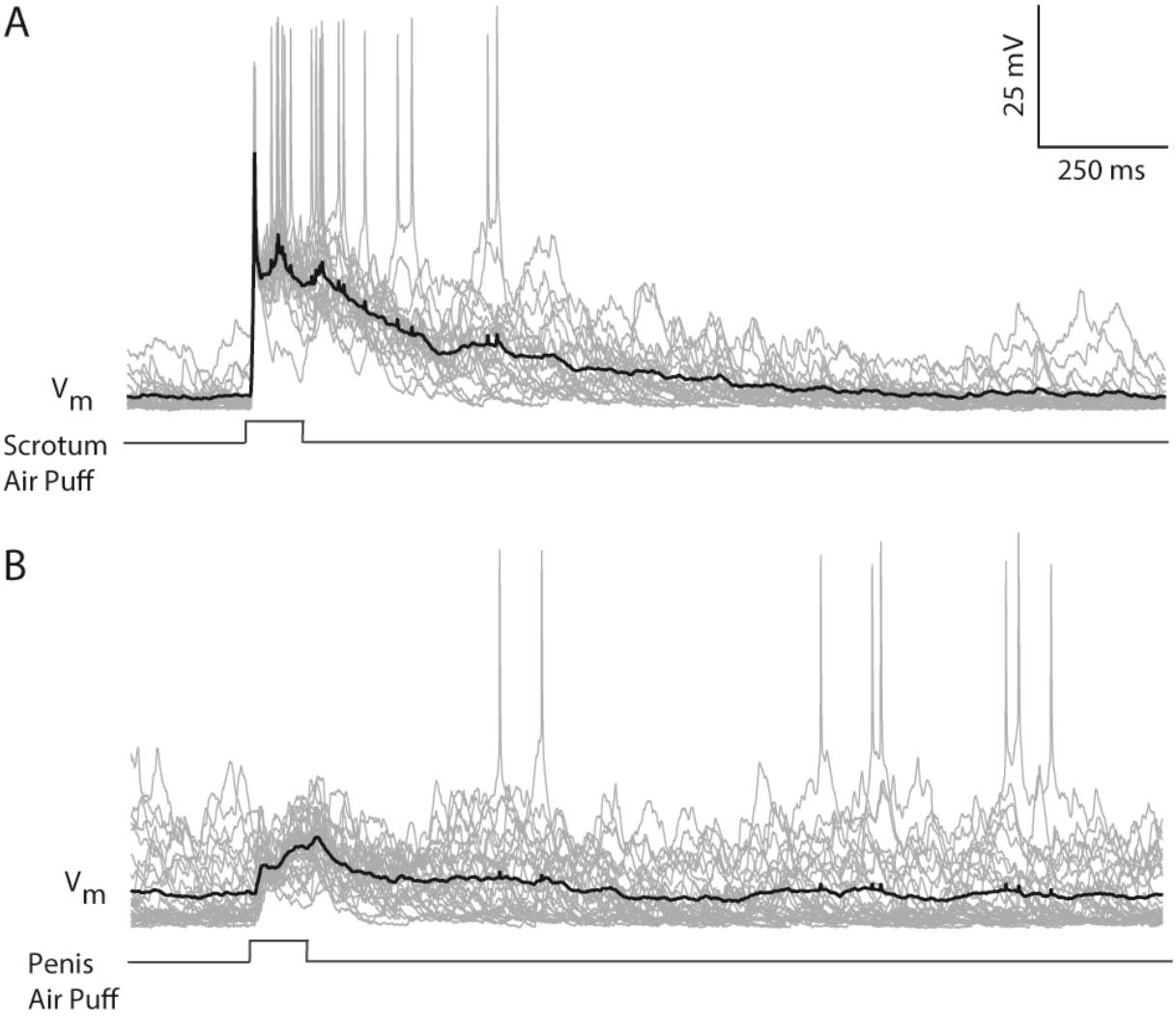


Figure S2: *In vivo* whole-cell recordings in genital cortex

In vivo whole-cell recordings from a superficial layer (depth 670 µm below the pia) genital cortex neuron.

(A) Scrotum stimulation. Grey traces represent the membrane potential in single trials. Top black trace shows the superimposed response average (20 trials). Lower black trace indicates the stimulus time course.

(B) Same as **A**, but for penis stimulation.

The recording allows two major observations: (1) The response amplitude to scrotum stimulation is very large confirming a genital representation in the somatosensory cortex. (2) The response to penis stimulation is markedly weaker, even though the air-puff sites were less than 1 cm apart. This observation indicates that receptive field in the genital area is small. The recording was obtained under urethane anesthesia at 2 mm posterior 2.5 mm lateral from bregma.

Figure S3. Related to Figure 4.

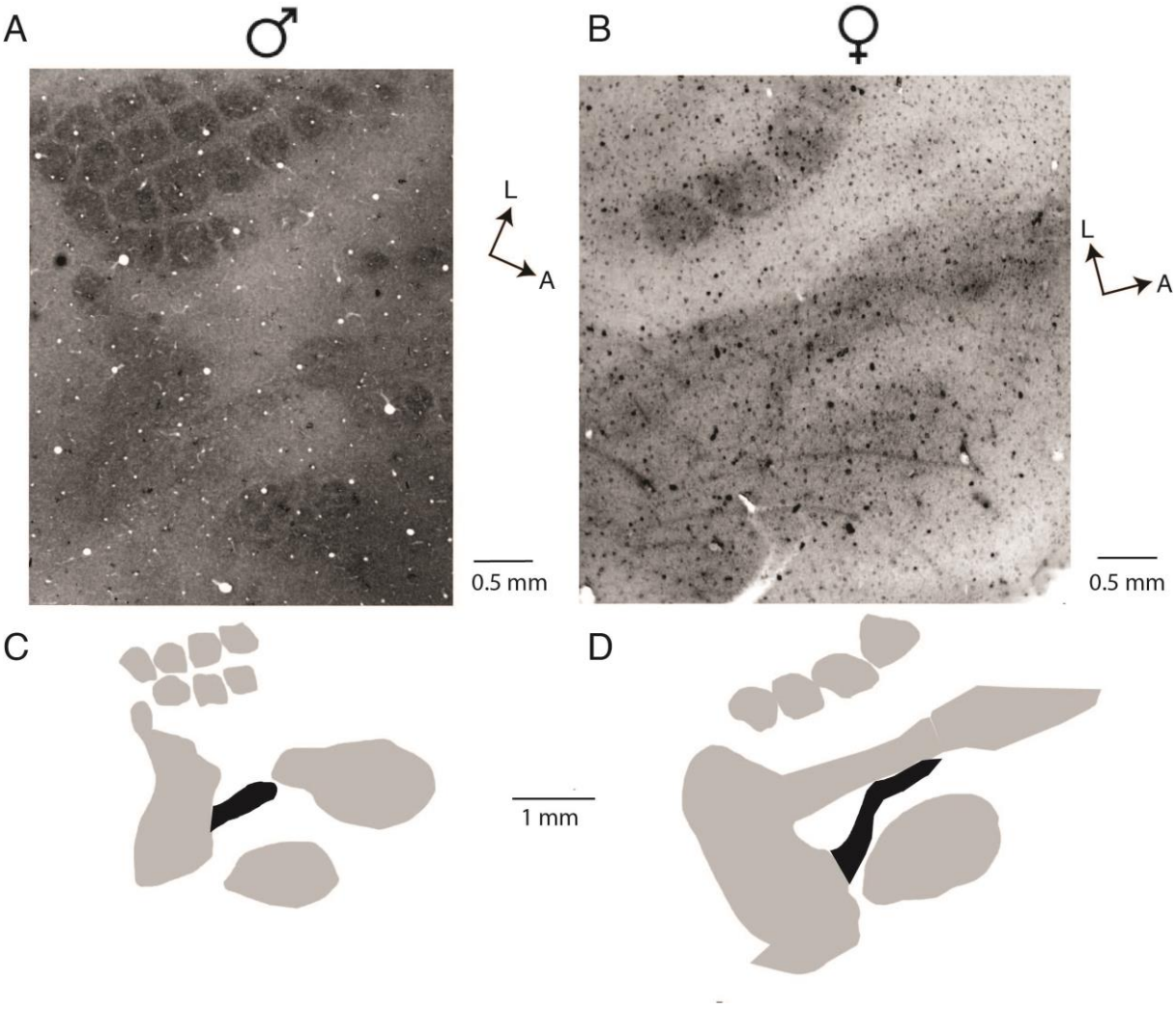


Figure S3: Cytochrome oxidase maps of genitals in somatosensory cortex of adult animals

- **(A)** Cytochrome oxidase staining of a tangential section through layer 4 of somatosensory cortex of a 6 week old male. A = anterior, L = lateral.
- **(B)** Somatosensory cortex of a 6 week old female, conventions as in **A**.
- **(C)** Drawings of male granular cortex body (gray) and granular penis (black) representations in two male rats; top drawing refers to **A**. Outlines were drawn only for the one section, which best (and completely) represented the genitals, body and trunk are partially incomplete.
- **(D)** Drawing of two female cortical body (gray) and clitoris (black) representations; top drawing refers to **B**, conventions as in **C**.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Animal welfare

All experimental procedures were performed according to German and American regulations on animal welfare and were approved by ethics committees in Berlin, Germany, and Woods Hole, MA, respectively. Long-Evans rats were provided by the Marine Biological Institute, Woods Hole (USA). Prepubescent animals for histological and physiological mapping were aged between post-natal day (P) 23 and P30. Adult animals for histological analysis were between 6 weeks and one year old. All animals were kept on a 12h:12h normal light/dark cycle with lights off at 8:00 p.m. Rats had ad libitum access to food and water.

Animals, surgery, receptive field mapping, whole-cell and single-unit recording

Long-Evans rats (P22–P30, n = 11, 6 males and 5 females) were anesthetized using urethane (1.4 g/kg, i.p.). Incised tissue was locally anesthetized with lidocaine. A rectal probe monitored body temperature and a homeothermic blanket (FHC) maintained it at 37 ± 0.5°C. For cortical mapping of the primary somatosensory cortex, an approximately 5 x 5 mm sized craniotomy was made 5 mm posterior to and 5 mm lateral to bregma. At each recording site we searched for clear tactile responses at a depth between deeper layer 3 (600 μm) to upper layer 5 (1300 μm) and plotted receptive fields. Electrode position was controlled by micromanipulators (Luigs & Neumann), which were set to 0 at the point of bregma. The first penetration was then made at 0.5 mm posterior and 2.5 mm lateral from bregma. The recording pipette was moved in 0.5 mm steps over the craniotomy until the full size of the craniotomy was penetrated. To improve mapping precision for the cortical genital area, we reduced the penetration spacing to 0.25 mm steps in case we encountered genital responses. Extracellular recording and hand mapping of receptive fields were performed in the left hemisphere of 6 males and 5 females with a 1 MΩ sized glass electrode. Voltage signals were amplified, differentially filtered for spikes, and sent to an audio monitor using a patch-clamp amplifier (Dagan) in current-clamp mode. Animals were positioned such that easy access to the genital region was possible. Specifically, the stereotaxic frame was close to the opening of the Faraday cage, the animal's head faced rightwards, the right body side (i.e. the side contralateral to the left hemisphere, which we mapped) was facing the experimenter. Animals were elevated with feet hanging down from a supporting plastic brick below the anterior body half. In this arrangement posterior parts of the trunk were accessible from all side; anterior ventral parts of the trunk were less well accessible.

Receptive fields were hand-plotted by systematically palpating the animal's body surface. Stimulation of the body surface was done in two different ways. For all body parts beside the vulva we used a short metal bar with which we applied fast gentle (resulting only in little skin indentation) strokes. The vulva was also stimulated with a thin (1 mm diameter) metal wire, with which we systematically stimulated internal parts of the vulva/clitoris in females.

In all receptive fields close to the animal's midline, we performed bilateral stimulation of the respective skin areas. Bilateral (midline-crossing) receptive fields were rare, however, and purely ipsilateral receptive fields were not observed.

Whole-cell recordings are obtained as described in [S1]. Pipettes were pulled to 3-8 MΩ (P1000, Sutter Instruments, Novato, Calif., USA) from filamented (0.25mm) borosilicate glass (OD 2.0mm, ID 1.5mm, Hilgenberg, Malsfeld Germany). Intracellular solutions were composed of (in mM): K-gluconate 130, Na-gluconate 10, HEPES 10, phosphocreatine 10, MgATP 4, GTP 0.3, NaCl 4 and biocytin 0.3–1% at pH 7.2. Signals were amplified (Cornerstone-amplifier, Dagan Corporation, Minneapolis MN USA), filtered at 3-10 kHz and digitized at 20 kHz (ITC-16; Instrutech, New York, N.Y., USA) using HEKA (Lambrecht, Germany) software. Recordings were exported and analyzed in Matlab 2014a (Natick, Massachusetts, USA).

For single-unit recordings we used 5 Mega Ohm glass pipettes and recorded large (> 0.5 mV) spikes of individual cells in the juxtacellular configuration on a Dagan-amplifier. These recordings were performed in 4 male and 3 female prepubescent rats. Recording traces were exported and analyzed in Matlab 2014a (Natick, Massachusetts, USA).

Histology

After physiological mapping, animals received an overdose of the anesthetic (20% urethane solution) and were perfused with phosphate buffer followed by a 4% paraformaldehyde solution (PFA). For anatomical maps, animals were anaesthetized and perfused in the same way. Brains were removed, hemispheres were separated, and cortices were flattened between two glass slides separated by clay spacers. Glass slides were weighed down with small ceramic weights for ~3 h. Flattened cortices were then stored overnight in 2% PFA and 100 μm sections were cut on a Vibratome (Leica). Sections were stained for cytochrome-oxidase activity using the protocol of Wong-Riley [S2]. Subsequently, pictures were taken on a Leica M165 FC microscope and outlines of granular somatosensory regions (indicated by a dark precipitate from the cytochrome oxidase stain) were drawn with ImageJ software.

Quantification of somatosensory areas and sizes

The area of various somatosensory regions were measured by outlining the anatomical region of interest and calculating its area using the ImageJ area calculating tool. The area of the following cortical representations were measured: hind-paw, forearm, trunk, penis, and clitoris. In addition, various aspects of the size of the cortical genital region were determined by measuring the length from the tip of the genital representation to its base (shaft length), the width half way from the base (half-width), and the length from the tip to the back of the trunk (total genital length) (see Figure 2C). Complete maps (Figure 4) were drawn by tracing body outlines and barrels through multiple serial tangential cortical sections using a computer-aided (Neurolucida) drawing system.

Matching anatomical maps to physiological response maps

The anatomical maps were matched to the physiological mapping using three different methods, which all led to the same conclusions. First, the individual recording sites were matched to anatomical map locations by placing electrolytic lesions. Lesions were placed by injecting 10 μA negative current through a tungsten electrode for 10 s. Second, individual physiological maps were matched to the overall layout of individual anatomical maps (n = 10). Third, anatomical maps (n = 17 hemispheres/maps from young animals and n = 9 hemispheres/maps from adult animals) were matched to our overall maps (Figure 1E and 1F) and published maps [S3, S4].

SUPPLEMENTAL REFERENCES

S1. Margrie TW, Brecht M, Sakmann B (2002) *In vivo* low resistance whole-cell recordings from neurons in the anaesthetized and awake mammalian brain. Pflügers archive-Eur. J Physiol *444*, 491-498.

S2. Wong-Riley, M. (1979). Changes in the visual system of monocularly sutured or enucleated cats demonstratable with cytochrome oxidase. Brain Research *171*, 11–28.

S3. Welker, C. (1971). Microelectrode delineation of fine grain somatotopic organization of SmI cerebral neocortex in albino rat. Brain research *26*, 259-275.

S4. Chapin, J.K., and Lin, C.S. (1984). Mapping the body representation in the SI cortex of anesthetized and awake rats. J. Comp. Neurol. 229, 199-213.

Lenschow et al. Cover-page suggestion

Legend:
Picture of Priapus – a Greek fertility god – from a Pompeian fresco superimposed on a rendition of a body map from rat somatosensory cortex. The erected posture of the penis points to a role of genital cortex in sexually aroused states. This conclusion is also suggested by the response properties of neurons in genital cortex. The illustration by Shimpei Ishiyama shows a modified Priapus photograph by Carole Raddato. For details see Lenschow et al. pages XX this issue.