Complex three-dimensional rearing environments amplify compensatory plasticity following early blindness

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SUMMARY

The neocortex has a remarkable capacity to alter its functional organization and connectivity in response to sensory loss, particularly if this loss occurs early in life. A key question is whether this cross-modal reorganization is driven by sensory deprivation itself or through enhanced use of the spared senses. We investigated how different rearing environments shape neural responses in primary somatosensory cortex (S1) of short-tailed opossums (*Monodelphis domestica)*, following elimination of visual inputs through bilateral enucleation in early development. Early blind and sighted littermates were reared in enriched environments to promote active tactile exploration in three-dimensional (3D) space, or in highly restricted standard laboratory cages. In adulthood, both enriched groups showed adaptive changes in exploration patterns and gap crossing behaviors relative to standard-reared counterparts. Thus, early blind animals showed behavioral compensation for vision loss, when challenged by complex environments. Enriched rearing increased selectivity of S1 neural responses to whisker touch and powerfully altered receptive field shapes such that they were less horizontally anisotropic. This shift was strongest in enriched early blind animals, enhancing tuning along the horizontal axis more than standard-reared early blind animals. Thus, alterations in S1 receptive fields following early blindness were amplified by environmental complexity. Sighted opossums reared with enrichment also showed similar whisker receptive field plasticity, though to a slightly lower degree. Together, these results demonstrate the strong influence of the rearing environment on reorganization of cortex that processes inputs from the spared senses, underscoring the role of experience in directing compensatory plasticity following early sensory loss.

INTRODUCTION

Early loss of vision leads to heightened perceptual abilities in the remaining senses, accompanied by structural and functional changes in the nervous system (Goldreich and Kanics, 2003; Wong et al., 2011; Voss, 2011; Morland et al, 2021; see Mezzera and López-Bendito, 2016; Lazzouni and Lepore, 2014 for review). However, the developmental origins of these changes remain poorly understood—it is not clear to what extent sensory experience, movement options and affordances contribute to compensatory changes in brain and behavior following early sensory loss. In this study, we investigate how enhancing tactile experience through rearing in an enriched environment contributes to the neural and behavioral plasticity of whisker–mediated touch in a marsupial, the short-tailed opossum, when vision is lost very early in development (Ramamurthy and Krubitzer, 2018; Ramamurthy et al., 2021).

It is well established that exposure to enriched environments drives plasticity in sensory cortices of juveniles, and even in adult animals with intact brains and bodies. This includes the functional reorganization of sensory representations through changes in cortical territory, functional properties (latency, strength, and selectivity of neural responses), and the morphology and molecular expression patterns of neurons (Seo, 1992; Leah et al., 1995; Coq and Xerri, 1998; Cancedda et al., 2004; Engineer et al., 2004; Polley et al., 2004; Guic et al., 2008; Mégevand et al., 2009; Devonshire et al., 2010; Alwis and Rajan, 2013, LeMessurier et al., 2018, Bibollet-Bahena 2023). Enrichment also counteracts degradation of representations associated with a transiently deprived sensory modality, and promotes recovery from brain injury (Polley et al., 1999; Bartoletti et al., 2004; Sale et al, 2007; Nakashima and Dyck, 2008; Wang et al., 2013;

Greifzu et al., 2014; Zhu et al., 2014; Zheng et al., 2014; Jiang et al., 2015; Kalogeraki et al., 2017; Levine et al., 2017). Enrichment through naturalistic experiences in accordance with a species' lifestyle engages animals in an ethologically relevant manner (Landers et al., 2011; Wilkinson et al, 2010; Branchi et al., 2011; Weissbrod et al, 2013) and is especially effective in reshaping cortical sensory representations at structural, functional and molecular levels (Polley et al., 1999; Polley et al., 2004; Frostig, 2006; Gomez-Pinilla et al., 2011; Landers et al., 2011). Therefore, in this study, we used a complex 3D rearing environment designed to mimic the natural habitat and lifestyle of short-tailed opossums.

Many small mammals, including short-tailed opossums, exhibit a continuum in patterns of vertical use of strata in a forest habitat, from the forest floor to the tree canopy (Jenkins, 1974; Jones et al., 2003; Delciellos and Vieira, 2006; Abreu and Oliveira, 2014; Shapiro et al., 2014). Short-tailed opossums are primarily terrestrial hunters and foragers (Jones et al., 2003) but prefer nesting above the ground in rocky outcrops (Macrini, 2004), using arboreal runways like branches and logs to negotiate complex terrain (Lammers, 2007; Lammer and Gaunter 2008; Ladine and Kissel, 1994; Montgomery, 1980). Short-tailed opossums are skilled climbers (Saunders et al., 1995; Saunders et al., 1998; Wilkinson et al., 2010) despite lacking many of the morphological specializations observed in more arboreal species (Schmitt et al., 2010; Rupert et al., 2014; Goin et al., 2016; Thomas et al., 2016). While they exhibit some arboreal traits—including some degree of forelimb specialization and tail prehensility (Jones et al., 2003; Schmitt et al., 2010; Rupert et al., 2014; Thomas et al., 2016)—short-tailed opossums primarily rely on behavioral strategies to navigate and maintain balance on arboreal substrates (Lammers, 2004; Lammers, 2007; Lammers, 2009; Lammers and Biknevicius, 2004; Lammers et al.,

2006; Lammers and Gauntner, 2008). Whisker touch is critical for climbing in spatially complex 3D environments (Ahl, 1986; Arkley et al., 2017), since it guides gap crossing and forelimb placement (Arkley et al., 2014; Niederschuh et al., 2015; Arkley et al., 2017; Grant et al., 2018). Peripheral organization of the whisker system is closely associated with lifestyle, being more well-developed in arboreal than in terrestrial species (Beddard, 1902; Pocock, 1914; Lyne, 1958; Lyne, 1959; Muchlinski, 2013; Grant et al., 2017; Grant et al., 2018) – in short-tailed opossums, whisker layout is consistent with semi-arboreal and scansorial marsupial species (Ramamurthy and Krubitzer, 2016; Grant et al., 2013; Lyne, 1958; Lyne, 1959). Thus, while short-tailed opossums are highly capable of vertical utilization of the environment, doing so involves behavioral modifications based on sensory input, including tactile information (Lammers and Biknevicius, 2004; Lammers, 2009).

In prior work, we showed that early loss of visual inputs in short-tailed opossums (at P4, prior to the formation of retinogeniculate and thalamocortical pathways) enhances tactile acuity and alters neural coding of whisker inputs in primary somatosensory cortex (S1), with receptive fields exhibiting greater selectivity along the natural axis of whisker motion (Ramamurthy & Krubitzer, 2018). Loss of vision at this early developmental stage also leads to major reorganization of thalamic and cortical connections of somatosensory cortex relative to sighted animals (Dooley & Krubitzer, 2019). This raises a critical question: are alterations in sensory coding in somatosensory cortex contingent on the deprivation of visual inputs itself, which gives rise to the novel anatomical connections that may form the substrate for these functional changes? Or does tactile experience drive functional plasticity, and can enrichment amplify the compensatory neural and behavioral

plasticity observed following early blindness? Here, we implement rearing conditions that encourage naturalistic patterns of tactile exploration in a spatially challenging environment designed to increase reliance on whisker use. By comparing how this enriched environment shapes neural coding of whisker touch stimuli in S1 and whisker-mediated behavior in sighted and early blind animals, we interrogate the role of tactile experience in directing compensatory plasticity following early blindness.

RESULTS

Blind and sighted opossums both adapt behaviorally to enriched rearing environments

Following bilateral enucleation on postnatal day 4, opossum mothers and their litters, including sighted and blind pups, were transferred into a new standard or enriched home cage environment (**Figure 1A**; **Figure S1A-D**). Prior to P12, sensory experience in either environment was determined entirely by patterns of behavior of the mother, due to the obligate attachment of opossum pups. Juvenile animals continue to rely on the mother for both sustenance and transportation until weaning at P56, but with increasing independent exploration (**Figure S1E-F**). Opossum mothers always preferentially nested high above the ground (3/3 mothers of four enriched experimental litters; example in **Video S1**), so pups were forced to rely heavily on touch to navigate the enriched environment. By adulthood, both sighted and early blind animals were highly adapted to their environments (**Video S2-S3**). Overall patterns of exploration did not differ between sighted and blind animals (**Figure S2**; **Figure 1B-C**), indicating successful behavioral compensation by blind animals even in more spatially complex environments. When placed in a novel arena with the dimensions and basic layout of the enriched home cages,

enriched opossums showed increased exploration of the vertical space compared to those reared in standard cages ($p<0.001$, permutation test; **Figure 1D**). In a gap crossing task conducted in the dark, opossums across all experimental groups performed fewer successful crossings when gaps were large (**Figure 1E-F**, 12-14 cm; eSC: 0.17, eEB: 0.14, sSC: 0.08, sEB: 0.14; gap crossings per minute across all trials) vs. small (2-10 cm; eSC: 2.29, eEB: 2.51, sSC: 2.75, sEB: 3.07). Consistent with previous studies in small mammals (Hutson & Masterton 1986, Voigts et al., 2015, Arkley et al. 2017, Graham & Socha 2020, Toso & Diamond 2023), gap crossing was heavily dependent on the whiskers. All experimental groups failed to cross large gaps when whiskers were trimmed and performed fewer crossings per minute even for small gaps. Notably, enriched groups were more affected by whisker trimming compared to standard groups (gap crossings per minute after whisker trimming – eSC: 1.34, eEB: 1.60, sSC: 2.31, sEB: 2.56), indicating their increased reliance on whisker-based strategies. In general, for all experimental groups, time taken to successfully finish crossing was longer for large vs. small gaps (**Figure 1G**; linear mixed-effect model, $p<0.001$; $p<0.001$ for all pairwise comparisons, permutation test), with small but significant increases in crossing times for small gaps following whisker trimming (linear mixed-effect model, $p<0.05$; sSC: $p<0.05$, sEB: $p<0.001$, eSC: $p<0.001$, eEB: $p<0.05$, permutation test). Differences between groups based on blindness or rearing conditions were most apparent for the duration to successfully cross large gaps. Early blind opossums were faster than sighted controls even under standard rearing conditions (sSC vs. sEB: $p<0.05$, permutation test), and their behavioral proficiency was amplified by enriched rearing (eEB vs. sEB: $p<0.001$, permutation test). Both sighted and blind enriched groups were significantly faster than

158 standard-reared animals ($p<0.001$ for all pairwise comparisons, permutation test) but not
159 significantly different from each other ($p>0.05$).

160 **Tactile experience amplifies compensatory neural plasticity in blind opossums**

161 Single-unit recording experiments were performed in 19 opossums reared in
162 standard conditions (**Figure 2**; 10 sSC, 9 sEB), and 14 opossums reared in enriched
163 conditions (**Figure 2**; 7 eSC, 7 eEB), acquiring data for a total of 339 neurons across
164 experimental groups (see **Table S1**; **Figure S3**). 63-73% of units recorded in S1 (sSC:
165 60, sEB: 64, eSC: 58, eEB: 51) had a significant evoked response (>2 SD above baseline)
166 to stimulus onset following single whisker deflections, and these neurons were included
167 in receptive field analyses. Raster plots and peristimulus time histograms (PSTHs) for
168 whisker-responsive neurons in each group are shown in **Figure 2A-B**.

169 As reported previously in standard-reared animals (Ramamurthy and Krubitzer,
170 2018), spontaneous firing rates of S1 neurons were low for all experimental groups but
171 were not significantly different among experimental groups (**Figure 2C**; mean ± SEM,
172 sSC: 0.582 ± 0.083 spk/s, sEB: 0.613 ± 0.121 spk/s, eSC: 0.544 ± 0.079 spk/s, eEB:
173 0.503 ± 0.082 spk/s; linear mixed-effect model, $p>0.05$). However, responses evoked by
174 the stimulation of even the most preferred single whisker stimulus (best whisker; BW) in
175 enriched groups were suppressed in relation to stimulus-evoked firing rates in standard-
176 reared sighted opossums (**Figure 2D**; mean ± SEM, sSC: 11.4 ± 0.839 spk/s, sEB: 8.39
177 ± 0.591 spk/s, eSC: 7.82 ± 0.748 spk/s, eEB: 4.57 ± 0.52 spk/s; linear mixed-effects
178 model, eSC vs. sSC: $p<0.01$, eEB vs. sSC: $p<0.001$; see **Table S2** for exact p-values for
179 the fixed-effect of experimental group, and for all pairwise comparisons between groups),

similar to previous observations in standard-reared early blind animals (for details see Ramamurthy and Krubitzer, 2018). Since relative suppression of BW-evoked responses was observed in enriched sighted controls, in addition to enriched early blind animals, this effect was not contingent on the lack of vision itself – rather it was driven by increased dependence on touch in the enriched groups of opossums. Mean BW-evoked firing rates in enriched sighted control animals were comparable to those in standard early blind animals (linear mixed-effects model, sEB vs. eSC: $p>0.05$). However, in enriched early blind animals, mean BW-evoked firing rates were lower relative to all other experimental groups (linear mixed-effects model, eEB vs. sEB: $p<0.001$, eEB vs. eSC: $p<0.001$, eEB vs. sSC: $p<0.001$). Thus, blindness was not required for suppression of whisker-evoked responses, but the combination of tactile enrichment and vision loss produced the greatest effect on whisker-evoked firing rates of S1 neurons. This is consistent with animals relying on their whiskers to a greater extent when reared in a complex environment in the absence of vision.

Average whisker receptive fields in both sighted and blind enriched groups were significantly smaller than those in standard-reared sighted controls (mean ± SEM, eEB: 3.22 ± 0.409, eSC: 3.48 ± 0.364, sSC: 5.92 ± 0.427; linear mixed-effects model, eSC vs. sSC: $p<0.001$, eEB vs. sSC: $p<0.001$) when measured as the number of whiskers that evoked a significant response above spontaneous firing (**Figure 2E**). We see similar results when receptive fields are quantified as the mean rank-ordered tuning curve across neurons (**Figure 2F**; linear mixed-effects model, eSC vs. sSC: $p<0.001$). The finding that tactile enrichment in sighted control animals leads to a decrease in mean receptive field size—similar to both standard-reared and enriched early blind animals—supports the

203 interpretation that this reduction reflects an experience-dependent effect across all three
204 groups. However, changes in measures of overall receptive field size in enriched early
205 blind animals relative to standard-reared early blind animals were either not significant
206 (**Figure 2E**, left, number of whiskers: eEB: 3.22 ± 0.409, sEB: 3.38 ± 0.270; linear mixed-
207 effects model, eEB vs. sEB: $p>0.05$) or modest (**Figure 2E**, right, rank-ordered tuning
208 curve: linear mixed-effects model, $p<0.01$). This suggests that, under standard rearing
209 conditions, receptive field plasticity in early blind animals may already have reached a
210 limit that cannot be further shifted by enrichment.

211 **Enrichment enhances selectivity for whisker touch along the behaviorally relevant**
212 **horizontal axis**

213 To better understand the effects of enriched rearing on receptive fields of S1
214 neurons, we characterized two-dimensional receptive fields for the row (horizontal) and
215 arc (vertical) dimensions of the whisker pad (**Figure 3A**). Visual inspection of mean 2D
216 receptive fields revealed clear differences in receptive field anisotropies across
217 experimental groups (**Figure 3B**). We quantified anisotropies in receptive fields as the
218 *shape index*, ranging in value from -1 to 1, where positive values indicated horizontally
219 anisotropic shapes, negative values indicate vertically anisotropic shapes and values
220 close 0 indicate more isotropic shapes. Receptive fields for neurons in the S1 whisker
221 representation in standard-reared sighted control and early blind animals were dominated
222 by horizontally anisotropic shapes (**Figure 3C-D**). However, for early blind animals reared
223 in enriched conditions, the receptive field shape distributions were shifted away from
224 horizontal anisotropy – either when considering only 1° surround whisker (SW) positions
225 (**Figure 3C**; neurons with shape index > 0.0, eEB: 37.3%, sSC: 68.3%, sEB: 57.8%; linear

mixed-effects model, eEB vs. sSC: $p<0.001$, eEB vs. sEB: $p<0.05$) , or when including all SW (1° - 3°) positions (**Figure 3D**; neurons with shape index > 0.0, eEB: 37.3%, sSC: 81.7%, sEB: 76.6%; linear mixed-effects model, eEB vs. sSC: $p<0.001$, eEB vs. sEB: $p<0.001$).

Receptive field distributions were also shifted away from horizontal anisotropy in sighted control animals reared in the enriched environment. This shift was not seen when only 1° SW positions were considered (**Figure 3C**; neurons with shape index > 0.0, eSC: 55.2%; linear mixed-effects model, eSC vs. sSC: $p>0.05$, eSC vs. sEB: $p>0.05$) but becomes apparent when 1° - 3° SW positions are considered (**Figure 3D**; neurons with shape index > 0.0, eSC: 46.6%; linear mixed-effects model, eSC vs. sSC: $p<0.05$, eSC vs. sEB: $p>0.05$). Thus, rearing in an enriched environment, which was permissive of more naturalistic patterns of tactile behavior, shifted the shapes of receptive fields of S1 neurons away from the horizontally anisotropic shapes typically found in animals reared in standard laboratory cages. The shift occurred in both sighted and early blind enriched animals, but the effect was more pronounced in early blind animals.

The results of receptive field shape analyses pointed to differential effects of rearing environment on tuning along horizontal and vertical axes of receptive fields. We quantified neuronal tuning width separately along whisker rows and arcs for each experimental group (**Figure 3E**). Both sighted and early blind enriched animals showed sharper tuning along both the vertical and horizontal axes relative to sighted controls. But there was a striking difference in the magnitude of tuning width changes along the horizontal vs. vertical axes. This is an amplification of the shift in receptive field anisotropies seen in early blind animals under standard rearing conditions (Ramamurthy

and Krubitzer, 2018). Along the arc (vertical) axis, tuning was sharpened to the same extent in the enriched groups as seen in standard-reared early blind animals relative to sighted controls (**Figure 3E**; %BW response for in-arc whiskers: eEB: 19.4 ± 2.80%, eSC: 18.7 ± 2.13%, sEB: 20.1 ± 1.58%, sSC: 26.7 ± 2.50%; linear mixed-effects model, eEB vs. sSC: $p<0.05$, eEB vs. sEB: $p>0.05$, eSC vs. sSC: $p<0.05$, eSC vs. sEB: $p>0.05$). But along the row (horizontal axis), both sighted and early blind enriched animals showed sharper tuning relative to both groups of standard-reared animals (**Figure 3E**; %BW response for in-row whiskers: eEB: 13.8 ± 2.07%, eSC: 20.9 ± 1.96%, sEB: 35.1 ± 2.82%, sSC: 49.1 ± 2.85%; linear mixed-effects model, eEB vs. sSC: $p<0.001$, eEB vs. sEB: $p<0.001$, eSC vs. sSC: $p<0.001$, eSC vs. sEB: $p<0.001$). There was a trend for sharper row tuning in enriched early blind animals compared to enriched sighted control animals, but this was not significant (linear mixed-effects model; eSC vs. eEB: $p>0.05$). We computed the whisker selectivity index as the normalized difference ratio of the neuronal response to the best whisker in comparison to responses to in-row or in-arc whiskers. Population distributions of the selectivity index for S1 neurons (**Figure 3F**) further validate the anisotropy in the enhancement of selectivity seen for animals reared in enriched environments (BW vs. in-arc whiskers: linear mixed-effects model, eEB vs. sSC: $p<0.05$, eEB vs. sEB: $p>0.05$, eSC vs. sSC: $p<0.05$, eSC vs. sEB: $p>0.05$, eEB vs. eSC: $p>0.05$; BW vs. in-row whiskers: linear mixed-effects model, eEB vs. sSC: $p<0.001$, eEB vs. sEB: $p<0.001$, eSC vs. sSC: $p<0.001$, eSC vs. sEB: $p<0.001$, eEB vs. eSC: $p>0.05$).

Thus, a spatially complex enriched environment drives significantly greater improvement in neuronal selectivity for whisker touch in early blind animals especially along the rostrocaudal axis, which is the primary axis of natural whisking behavior. A

similar improvement in selectivity was observed in sighted control littermates reared in the enriched environment, supporting the role of tactile experience rather than vision loss itself in driving these effects. In summary, the results of our current study indicate that compensatory effects on neural coding in S1 following early blindness do not arise solely from visual deprivation and can be amplified and directed by tactile experience.

DISCUSSION

Although the effects of enrichment on plasticity within a sensory modality have been studied at structural, functional, and molecular levels, few studies have examined its impact on cross-modal compensatory plasticity, focusing mainly on c-fos expression or synaptic changes (Piche et al., 2004; Mundiñano and Martínez-Millán, 2010; Zheng et al., 2014). Here, we tested the effects of rearing sighted and early blind short-tailed opossums in a large and dynamic, spatially complex 3D environment, and examined effects on on tactile behavior and receptive field configurations of neurons in S1.

Enrichment reduces abnormal stereotypic behaviors (e.g. overgrooming) and other behavioral indicators of stress, aggression, and enhances learning, memory, motor performance and social behavior (Dijkhuizen et al., 2024; Ratuski et al., 2024; Cait et al., 2024; Nakhwa et al., 2024; Caires & Bossolani-Martins, 2023; Mieske et al., 2022; Kentner et al., 2021; Khoo et al., 2020; Bailoo et al., 2018; Girbovan & Plamondon, 2013; Amaral et al., 2008; Mason et al., 2007). It can also improve sensory function and performance, including whisker touch discrimination (Zheng et al., 2021). In our study, we implemented enrichment intended to increase the use of whisker touch. Since whiskers are critical for guiding forelimb placement in crossing gaps when small mammals climb (Ahl, 1986; Arkley et al., 2014; Niederschuh et al., 2015; Arkley et al., 2017; Grant et al.,

295 2018), we reared opossums in cages with periodically varying, spatially complex climbing
296 substrates and intermittently introduced novel toys, since novel objects and other
297 interactive enrichment devices promote active whisking behavior (Nakhwa et al., 2024;
298 Sofroniew & Svoboda, 2015). We show that in a novel setting, both sighted and blind
299 opossums showed differential exploration patterns reflecting the geometry of their rearing
300 environments. Additionally, enriched opossums are faster and more successful in gap
301 crossing using a whisker-dependent strategy. Thus, opossums use behavioral strategies
302 shaped by their rearing environments, and our enrichment approach effectively engages
303 and enhances naturalistic whisker-mediated behavior in both sighted and blind animals,
304 enabling us to evaluate the effects of enriched rearing on compensatory plasticity
305 following early vision loss.

306 We have previously shown that early blindness alters the coding of whisker touch
307 inputs in opossums, in a manner that supports enhanced tactile discrimination
308 (Ramamurthy & Krubitzer 2018, Ramamurthy et al., 2021). This involved suppression of
309 whisker-evoked responses combined with increased spatial selectivity, due to the greater
310 relative suppression of neural responses to non-preferred stimuli (SW) vs. the preferred
311 stimulus (BW). These alterations in the receptive fields of neurons in S1 resemble the
312 effects of naturalistic environmental enrichment on receptive fields of neurons in S1 of
313 adult rats, described by Polley and colleagues (1999, 2004). Here we demonstrate that
314 rearing early blind short-tailed opossums in an enriched environment further amplifies
315 these changes. Since selectivity to whisker stimuli was enhanced not just in early blind
316 animals but also in sighted control animals reared in enriched conditions, we can infer
317 that suppression of whisker-evoked responses and increased selectivity of neural

responses in the S1 whisker representation are effects of tactile experience rather than visual deprivation itself.

A key finding was the effect of the rearing environment on the shapes of receptive fields of S1 neurons – shape distributions for both sighted and early blind animals exhibited a prominent shift away from the horizontally anisotropic shapes that dominate in standard-reared opossums. This was accompanied by a reduction in tuning width along row (horizontal) vs. arc (vertical) axes in enriched animals – on average, tuning widths were reduced to a greater extent along the row axis, parallel to the main axis of whisker movements. This may reflect more naturalistic patterns of whisker use in short-tailed opossums, enabled by the increase in vertical space and availability of climbing substrates in the enriched rearing environment. In rats, simulations that modelled exploratory behavior predicted changes in the spatial patterns of whisker contact depending on the environment being explored (Hobbs et al., 2015) – whiskers within the same horizontal row were more likely to come in simultaneous contact during exploration of flat surfaces, but in more complex and naturalistic environments, the probability distributions of simultaneous whisker contact shifted away from this row-wise structure. Such alterations in the patterns of tactile stimuli impinging on the whisker array over the course of development may, in turn, impact receptive field structure.

Another possibility is that anisotropic effects on receptive fields are inherent to differences in row vs. arc organization within the neocortex. In rats and mice, it is well-documented that there is a bias towards preferential connectivity among barrels corresponding to whiskers in the same row (Bernardo et al., 1990; Hoeflinger et al., 1995; Kim and Ebner, 1999; Keller and Carlson, 1999), and neurons in barrel cortex typically

display patterns of activity that tend to be elongated along the row axis (Armstrong-James and Fox, 1987; Simons, 1978; Kleinfeld and Delaney, 1996; Ego-Stengel et al., 2005; Le Cam et al., 2011). Further, there is evidence for differential effects on responses of S1 neurons along row and arc axes when sensory inputs are altered within the whisker system, for example, through chronic stimulation (Quairiaux et al., 2007) or selective trimming (Dubroff et al., 2005; Wallace and Sakmann, 2008) of specific whiskers. Thus, biases in the functional organization and connectivity within the S1 whisker representation may favor plasticity, preferentially sharpening tuning along the row axis.

A long-standing question has been whether reorganization following sensory loss is primarily driven by deprivation of the lost modality or due to increased experience using the spared senses. Studies in blind humans with Braille reading experience support an important role for tactile experience in generating tactile spatial acuity enhancement following blindness (Wong et al., 2011). The relative contributions of deprivation of the lost modality versus increased or modified use of the spared senses (Goldreich and Kanics, 2003; Wong et al., 2011; Voss, 2011) to different aspects of cortical reorganization following sensory loss are still not well understood. Our study provides clear evidence for the role of experience in driving compensatory neural and behavioral plasticity following sensory loss, highlighting the importance of therapeutic strategies engaging tactile exploration (for example through play-based learning of tactile skills, Hilditch 2024; or the use of haptic devices, Fleck et al., 2025) to maximize neural and behavioral compensation through touch.

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368 **AUTHOR CONTRIBUTIONS**

369 D.L.R. and L.A.K. designed the study. D.L.R., M.E., T.J.K., and H.D. performed the
370 experiments. D.L.R. analyzed the data. D.L.R. and L.A.K. wrote the manuscript.

371 **DECLARATION OF INTERESTS**

372 The authors declare no competing interests.

373 **MATERIALS AND METHODS**

374 **Animals**

375 Thirty-three adult short-tailed opossums (*Monodelphis domestica*; age range: 4-16
376 months; weight range: 61-165g) were used for electrophysiological recording
377 experiments. Animals belonged to four experimental groups (**Table S1**) across two
378 rearing paradigms (standard: ‘s’, enriched: ‘e’). Data from animals in the standard-rearing
379 groups were reported in a previous publication (Ramamurthy and Krubitzer, 2018),
380 however, new analyses of some aspects of the data are additionally presented here for
381 comparison with data from enriched-rearing groups.

382 **Ethics Statement**

383 All protocols were approved by the Institutional Animal Care and Use Committee
384 of the University of California, Davis, and experiments were conducted according to the

385 criteria outlined in the National Institutes of Health *Guide for the Care and Use of*
386 *Laboratory Animals*.

387 **Bilateral enucleation**

388 For all experimental groups, bilateral enucleations were performed at P4 using
389 procedures previously described (Ramamurthy and Krubitzer, 2018). Mothers of
390 experimental litters were lightly anesthetized with Alfaxan (initial dose: 20 mg/kg;
391 maintenance doses: 10-50%; IM) to facilitate enucleation of the pups, which are attached
392 to the mother at this developmental stage. Pups were anesthetized by hypothermia.
393 Health of both the mother (respiration rate and body temperature) and the pups
394 (heartbeat, coloration and mobility) was monitored throughout the procedure. An incision
395 was made in the skin covering the eyes, the eyes were removed under microscopic
396 guidance, and the skin was repositioned and resealed using surgical glue. Approximately
397 50% of each litter was bilaterally enucleated. After complete recovery from anesthesia,
398 mothers along with their attached litters were placed into either standard or enriched
399 housing, depending on the experimental group.

400 **Rearing paradigms**

401 Mothers of all experimental groups were reared and housed in standard laboratory
402 cages until bilateral enucleations were performed on the pups at P4. Following the
403 enucleation procedure, mothers with litters belonging to the standard-rearing groups were
404 placed in standard laboratory cages, while those belonging to the enriched-rearing groups
405 were placed in custom-made enriched cages (**Figure S1**). All animals were maintained
406 on a 14/10 light/dark cycle. Food and water were available *ad libitum* in the cage under
407 both rearing conditions. All experimental litters were housed together with the mother until

408 weaning at ~8 weeks (P56), and at that stage they were separated from the mother, but
409 the whole litter continued to be housed together until adulthood at ~4 months (P120).
410 Adults (>P120) were individually housed under standard rearing conditions, but in groups
411 of 2-4 same-sex littermates under enriched conditions (see below).

412 Standard-rearing: Under standard-rearing conditions, adult animals were singly housed
413 in standard laboratory cages, in cages similar to those typically used for laboratory
414 rodents (cage dimensions: 18.5”l x 10”w x 8”h). The home cage was provided with a
415 nesting cup and nesting material (shredded paper towels).

416 Enriched-rearing: The enriched rearing environment in our study incorporated features
417 common to environmental enrichment (EE) paradigms previously used in rodents
418 (Nithianantharajah and Hannan, 2006; Sale et al., 2009; Bengoetxea et al., 2012), and
419 additionally takes into consideration the natural lifestyle of short-tailed opossums (**Figure**
420 **S1**). Juvenile animals were cohoused until weaning with the mother as well as littermates,
421 and then with just their littermates from weaning until adulthood, as described above.
422 Enriched cages were provided with standard bedding and nesting material, as well as
423 some additional nesting material with a different texture. In addition to the nesting cup
424 placed on the floor as in standard cages, enriched cages provided the option of nesting
425 high above the cage floor, in nesting boxes attached to the cage walls. A single nesting
426 box was provided in the cage at juvenile stages of the experimental litters. In adulthood,
427 balanced numbers of eSC and eEB littermates were housed together in each enriched
428 cage, with a maximum of four opossums per cage. Each adult animal was provided an
429 individual nesting box. Enriched cages were substantially larger (~8x in volume; 29”l x
430 18”w x 24”h) compared to standard cages to encourage tactile exploration in all three

431 dimensions. The greatest increase relative to standard cages was along the vertical
432 dimension: enriched cages were 3x taller than standard cages. Enrichment toys (Bio-
433 Serv) of different shapes and textures were provided in the cage. Toys were replaced,
434 and the positions of manzanita branches were changed every 2–3 days to introduce
435 novelty and promote continual exploration of the environment. .

436 **Behavioral testing**

437 Arena testing: Arena testing was performed in three adult animals from each of the four
438 experimental groups. The arena consisted of a cage with the dimensions of enriched
439 cages, containing a single nest box near the top of the arena, with a branch leading up to
440 it. Animals were placed in the arena and allowed to explore it for 5 minutes in the
441 darkness, and videos of their behavior were acquired under infrared (IR) illumination.
442 idTracker software (**Figure S2A**; Romero-Ferrero et al., 2018) was used to extract
443 movement trajectories of individual animals, which was further analyzed in Matlab.
444 Exploratory activity for each animal was quantified as the average number of frames in
445 which they spent in the regions defining horizontal and vertical space within the arena,
446 and the vertical exploration index was calculated as:

447 $$Vertical\ Exploration\ Index = \frac{(Vertical\ exporation - Horizontal\ exploration)}{(Vertical\ exporation + Horizontal\ exploration)}$$

448 Thus, more positive values indicated a shift towards vertical exploration, while more
449 negative values indicated a shift towards horizontal exploration.

450 Gap crossing task: Gap crossing was tested in 3-4 adults animals per experimental group.
451 Animals were allowed to spontaneously cross from one platform ("start") to another

452 platform (“goal”) with either small (2-10 cm) or large gap distances (12-14 cm) separating
453 them, which were interleaved in a pseudorandom order. Animals were tested for 7 days
454 with intact whiskers, followed by 1-3 days of testing after whisker trimming. On each trial
455 animals were allowed to remain on the start platform and attempt crossing for a maximum
456 of 1 minute. If no crossings were performed, the trial was ended and animals were
457 removed from the platform for 1 min before returning to the platform for the next trial.
458 Videos of this behavior were acquired under IR illumination or dim red light, and
459 DeepLabCut was used for analysis of gap crossings (**Figure S2B-C**). The snout tip, the
460 base of the tail and the position of the edges of each platform were labeled in a subset of
461 frames to generate the dataset (260 frames across 13 opossums) on which the network
462 was trained (200,000 iterations). On a subset of trials, slow-motion video clips in a
463 zoomed-in field of view were acquired to visually confirm the use of whiskers during gap
464 crossing. Infrared motion sensors (emitter/detector pairs) placed on either side of the
465 edge of each platform detected beam breaks and beam break times were recorded as a
466 secondary method of confirmation.

467 **Surgical procedures**

468 Animals were anesthetized with urethane (initial dose: 1.25 g/kg, 30% in saline, IP;
469 supplemental doses: 0.125-0.313 g/kg, 30% in saline, IP). Vital signs (respiration and
470 body temperature) were monitored throughout the experiment. At the beginning of the
471 surgery, dexamethasone (0.4-2.0 mg/kg; IM) was administered to minimize intracranial
472 swelling. Lidocaine (2% solution; SC) was injected down the midline of the scalp and
473 around the ears. The animal was then placed in a stereotaxic frame. Following an incision
474 at the midline of the scalp and retraction of the temporal muscle, a craniotomy was

475 performed to expose the entire parietal cortex and the dura was retracted. The surface of
476 the neocortex was covered with a layer of silicone fluid to prevent desiccation and then
477 photographed so that electrode penetration sites could be related to patterns of
478 vasculature. The head of the animal was stabilized by cementing a head post to a skull
479 screw inserted rostral and contralateral to the craniotomy.

480 **Extracellular recordings and whisker stimulation**

481 Somatosensory receptive fields were first coarsely mapped by recording multiunit
482 activity while applying tactile stimuli using a handheld probe. The representation of the
483 whiskers in S1 was localized by progressively testing electrode penetration sites medial
484 to the rhinarium representation and caudal to the lower jaw/lip representation. After this,
485 when receptive fields were found to be located on the mystacial or genal whisker pad (),
486 computer-controlled whisker deflections were used quantitatively measure single unit
487 receptive fields.

488 Whisker stimuli consisted of single whisker deflections (2°) delivered using
489 piezoelectric actuators (4 ms/100 ms/4 ms ramp-hold-return; 5 mm from the whisker
490 base; 1.4 mm forward excursion). Data were collected for a grid of sixteen whiskers on
491 the mystacial pad to enable construction of somatotopic receptive fields. Whiskers were
492 deflected in a pseudorandom order with 50-100 trials collected per whisker (in blocks of
493 3-50 trials) at each recording site. Extracellular recordings (amplifier gain: 10,000X; A-M
494 Systems Model 1800 Microelectrode AC Amplifier; A-M Systems, Carlsborg, WA;
495 sampled at 28 kHz; Power1401, Cambridge Electronic Design Limited, Cambridge, UK)
496 were made 400-500 μm below the pial surface using tungsten microelectrodes (FHC,
497 Inc., Bowdoin, ME; 1–5 MΩ at 1 kHz) lowered using a hydraulic microdrive (David Kopf

498 Instruments, Tujunga, CA). Recordings were bandpass filtered (300-3000 Hz), and spike-
499 sorting was carried out offline to confirm isolation of single units.

500 After electrophysiological data was collected, fluorescent probes were used to
501 mark the location of recording sites, so that these could be related to histologically
502 identified cortical field boundaries (**Figure S3**).

503 **Histology**

504 At the end of each experiment, animals were injected with an overdose of sodium
505 pentobarbital (Beuthanasia; 250 mg/kg IP) and transcardially perfused with 0.9% saline,
506 followed by 2-4% paraformaldehyde in phosphate buffer, and then 2-4%
507 paraformaldehyde in 10% phosphate-buffered sucrose. The brain was extracted and
508 post-fixed (1-2 hours in 4% paraformaldehyde in 10% phosphate-buffered sucrose). In
509 some cases, the cortical hemispheres were flattened and the tissue was sectioned
510 tangentially at 30 μm using a freezing microtome and processed for myelin staining
511 (Gallyas, 1979; Dooley et al, 2013) to enable reconstruction of cortical field boundaries.
512 Reconstructions were drawn using in Adobe Illustrator CS5 (Adobe, San Jose, CA).
513 Digital images of processed tissue were obtained using either a Nikon Multiphot system
514 or an Optronics MicroFire digital microscope camera. Contrast and brightness of whole
515 images were adjusted using Adobe Photoshop CS5 (Adobe, San Jose, CA).

516 **Neural data analysis**

517 Data files were processed blind to experimental group identity. Single-unit isolation
518 was performed while running the experiment using template-matching procedures in
519 Spike2 (Cambridge Electronic Design Limited, Cambridge, UK; RRID:SCR_000903), and

520 unit isolation was verified offline in Spike2, using principal component analysis. Stable
521 waveforms and firing rates over the course of the recording session, and <0.5% refractory
522 period violations (interspike interval <1.5 ms) were required for units to be included in the
523 analysis. Spikes times were then exported to MATLAB (Mathworks, Inc., Natick, MA;
524 RRID:SCR_001622) and analyzed using custom scripts. Whisker evoked firing rates were
525 measured as the spike count following stimulus onset (0-100 ms) and were considered
526 significant if they exceeded prestimulus spike counts in an equivalent time window by two
527 standard deviations. The *best whisker* (BW) was defined as the whisker with the highest
528 evoked response, and depending on their proximity to the BW the remaining whiskers
529 were categorized as *first-order surround whiskers* (1° SW; immediately adjacent to BW),
530 *second-order surround whiskers* (2° SW; one position removed from BW) and *third-order*
531 *surround whiskers* (3° SW; two positions removed from BW).

532 One measure of receptive field size used was the number of whiskers that evoked
533 a significant response in each recorded neuron. Another measure used was the rank-
534 ordered whisker tuning curve for each neuron, where responses to all whiskers tested
535 were sorted in descending order of whisker-evoked response magnitude, and normalized
536 to peak (BW response). Further, mean 2D somatotopic receptive fields were obtained
537 after normalizing SW-evoked response magnitudes to the BW-evoked response, and
538 aligning receptive fields of single units such that the BW was at the center of the receptive
539 field for each unit. 50, 75, and 90% contour lines (for response levels relative to the BW)
540 were plotted for the mean receptive fields (smoothed by linear interpolation) to facilitate
541 visualization. Raw values were used for all calculations and statistical comparisons.

Anisotropies in the structure of somatotopic receptive fields were quantified as the *shape index:*

$$Shape\ index = \frac{(Row\ tuning\ width\ index - Arc\ tuning\ width\ index)}{(Row\ tuning\ width\ index + Arc\ tuning\ width\ index)}$$

If the sum of the row and arc tuning width indices was zero, shape index was assigned to be zero. Shape indices ranged from -1 to 1, with values >0 indicating broader tuning along the row compared to the arc (i.e. broader horizontal tuning), and values <0 indicating broader tuning along the arc compared to the row (i.e. broader vertical tuning).

Neuronal selectivity for the best whisker along the row and arc dimensions was measured by assessing tuning width separately along the horizontal and vertical axes as the *row tuning width index* and the *arc tuning width index* respectively, either considering only 1° SW, or 1°, 2°, 3° SW in the row or arc of the BW, as stated below:

$$Row\ tuning\ width\ index = \frac{(\text{Mean evoked response of } SW_{row})}{\text{BW evoked response}}$$

$$Arc\ tuning\ width\ index = \frac{(\text{Mean evoked response of } SW_{arc})}{\text{BW evoked response}}$$

Additionally, the *whisker selectivity index (WSI)* for BW vs. in-row or in-arc whiskers was computed separately for each neuron, considering BW responses relative to 1°, 2°, 3° SW responses.

$$WSI_{row} = \frac{(BW\ evoked\ response - SW_{row}\ evoked\ response)}{(BW\ evoked\ response + SW_{row}\ evoked\ response)}$$

$$WSI_{arc} = \frac{(BW\ evoked\ response - SW_{arc}\ evoked\ response)}{(BW\ evoked\ response + SW_{arc}\ evoked\ response)}$$

In-row and in-arc selectivity indices ranged from 0 to 1, with values closer to 1 indicating higher selectivity for the BW in the corresponding dimension.

Statistical Analysis

Permutation tests for the difference in means were used to assess differences in mean between two groups (referred to in brief as 'permutation test'). All statistical tests for neuronal data use 'n' of cells. We account for interindividual variability using a linear mixed effects model with the formula:

$$Response\ Variable \sim Experimental\ Group + (1|Sex) + (1|Animal\ ID)$$

where experimental group is modeled as a fixed effect, and sex and animal ID are modeled as random effects. For gap crossing behavior, an additional model was fit to test for fixed effects of test condition (large vs. small gaps, intact vs. trimmed whiskers):

$$Response\ Variable \sim Experimental\ Group + Test\ Condition + (1|Sex) + (1|Animal\ ID)$$

where experimental group and test condition are modeled as fixed effects, and sex and animal ID are modeled as random effects. Exact p-values for the fixed effect of experimental group and/or test condition in the linear mixed-effects model, and pairwise comparisons across experimental groups are provided in **Table S2.**

FIGURE LEGENDS

Figure 1. Behavioral differences in opossums reared in enriched environments. A. Timeline showing experimental manipulations relative to key milestones in the development of short-tailed opossums. Behavior and electrophysiology experiments were conducted in adults following the sequence shown in the inset, but ages were not

precisely matched to the exact day. **B.** Example movement trajectories from one animal in each experimental group (sEB: light red, eEB: dark red, sSC: light blue, eSC: dark blue) tracked over five minutes during behavioral testing in an enriched arena. **C.** Overlaid movement trajectories from all animals in each experimental group in the arena test show a pattern of increased exploration of vertical vs. horizontal space in opossums reared in enriched cages compared to those reared in standard cages. Plus symbols indicate center of mass of movement trajectories for each group (colors as in **B**), with data for animals combined within in each rearing condition in black. **D.** Mean vertical exploration index across animals. Animals reared in the enriched environement show significantly higher levels of vertical exploration. **E.** Experimental setup in the gap crossing task. **F.** Number of gap crossings per minute (across all trials) for large and small gaps (with intact or trimmed whiskers). Gap crossing was whisker-dependent in all groups. No opossums successfully crossed large gaps after whisker trimming, so this not plotted. **G.** Duration for successfully crossing large or small gaps (with intact or trimmed whiskers). All groups show significantly longer durations for succesful gap crossing on large gaps (12-14 cm) vs. small gaps (2-10 cm). Upon whisker trimming, all groups show a significant increase in gap crossing duration for small gaps, consistent with the use of whisker-dependent strategies for gap crossing, as seen in **(F)**. For larger gaps, there are prominent differences between experimental groups based on rearing condition and blindness. Early blind animals crossed gaps faster than sighted controls in standard conditions, and their performance was further enhanced by enriched rearing.

Figure 2. Enriched rearing amplifies alterations in S1 neuron responses to whisker touch in early blind animals, while also producing similar shifts in sighted controls.

604 **A.** Spike raster plots for example neurons responsive to whisker touch in each of the four
605 experimental groups (left to right, sSC, sEB, eSC, eEB). Examples are shown for neurons
606 with the same best whisker (BW), namely C2. Trials corresponding to A, B, C and D
607 whisker rows are separated by horizontal lines. Within each row the direction of more
608 rostral 'R' vs. more caudal 'C' whiskers is labeled on the y-axis.**B.** Peristimulus time
609 histograms (PSTHs) corresponding to the same neurons shown in **(A)**. PSTHs
610 corresponding to whiskers with response magnitudes >50% the BW response are in
611 black, others are in gray**.** The examples in **(A)** and **(B)** illustrate more selective responses
612 to whisker touch in standard-reared blind opossums, which is further amplified in enriched
613 groups. Additionally, neural responses to whiskers vertically adjacent to the BW are
614 stronger than those for horizontally adjacent whiskers in enriched groups, especially in
615 enriched early blind opossums. **C.** Spontaneous firing rates of S1 neurons were not
616 significantly different across experimental groups. **D.** Firing rates evoked by the BW
617 showed significant differences across experimental groups. Notably, both sighted and
618 early blind enriched groups showed suppression relative to standard-reared sighted
619 controls, similar to that which we previously described in standard-reared early blind
620 animals. The magnitude of suppression of the BW-evoked response was greatest in early
621 blind animals reared in the enriched environment. **E.** Mean receptive field size was
622 significantly smaller in sEB, eSC and eEB animals relative to standard-reared sighted
623 controls. This is seen both in terms of number of whiskers driving significant responses
624 (left) and the rank-ordered tuning curve (right) for whisker-responsive S1 neurons. See
625 also **Table S2**.

Figure 3. Enriched rearing amplifies anisotropies in receptive fields of S1 neurons in early blind animals, improving selectivity for whisker touch along a behaviorally relevant axis. A. Schematic diagram of the opossum mystacial whisker pad indicating whisker rows (light yellow) and arcs (gold), and the hortical and vertical axes used for shape analyses. The schematic shown as an inset in individual panels depicts the grid of 16 whiskers and the surrounding whisker (SW) positions considered in that particular analysis for one example BW position (black filled circle) on the grid. **B.** Mean 2D somatotopic receptive fields for each experimental group (left to right, sSC, sEB, eSC, eEB) with 50, 75, and 90% contour lines (for response levels relative to the BW, smoothed by linear interpolation for display). **C-D.** Receptive field shape distributions for S1 neurons in enriched animals were shifted away from horizontal anisotropic shapes, which are typical of standard-reared animals. **C** shows receptive field shape distributions when only 1° SW positions were considered (left) and fractions of horizontally anisotropic receptive fields corresponding to each group (right). Light gray bars indicate horizontally elongated receptive fields defined as shape indices > 0, while black bars indicate horizontally elongated receptive fields with a stricter cutoff of shape indices > 0.5. **D** shows data for the same neurons when all SW positions (1°-3°) were included. Horizontally anisotropic receptive fields are decreased in both enriched groups of animals, and to the greatest extent in enriched early blind animals. **E.** Quantification of tuning width along the row (horizontal/rostrocaudal axis) and arc (vertical axis) across experimental groups. Enriched groups show sharper tuning along the horizontal axis, to a significantly greater extent than standard-reared early blind animals. Enriched groups also show sharper tuning along the vertical axis compared with standard-reared sighted controls, but only to

649 the same extent as previously observed in standard-reared early blind animals. **F.**
650 Distributions of the whisker selectivity index of S1 neurons calculated separately for in-
651 arc whiskers (left) and in-row whiskers (right), when including all SW positions (1°- 3°).
652 sEB, eSC and eEB groups all showed significantly greater selectivity for whiskers within
653 arcs, but were not significantly different from each other. The greatest increases in
654 whisker response selectivity were seen for whiskers within rows i.e. the
655 horizontal/rostrocaudal axis, which is the primary axis of natural whisker motion.
656 Selectivity was greatest for eEB animals followed by eSC and sEB animals, with all three
657 of these groups showing significantly higher selectivity along the horizontal axis comapred
658 to sSC animals.

659 **SUPPLEMENTARY FIGURE LEGENDS**

660 **Figure S1. Standard and enriched rearing environment used for short-tailed**
661 **opossums. A.** Comparison of cages used in standard rearing (L) and enriched rearing
662 paradigms (R). Enriched cages (29”l x 18”w x 24”h) were much larger (~8x in volume)
663 than standard cages (8.5”l x 10”w x 8”h), especially in the vertical dimension (3x), and
664 provided with a nesting box high above the ground, in addition to the standard nesting
665 cup placed on the cage floor. They contained various enrichment objects: enrichment toys
666 that were regularly switched out and manzanita branches that were regularly switched in
667 position. They were also provided with a running wheel, and incoporated social housing
668 (see *Materials and Methods*). **B.** Frontal view of an enriched cage. **C.** Top view of the
669 same enriched cage as **(B)**. Letters indicate different components of the cage, which are
670 visible in both views: nesting box (a), nesting cup (b), food (c), water (d), running wheel
671 (e), manzanita branches (f-l), and enrichment toys (m). **D.** Mother with an attached

experimental litter after placement in the enriched rearing environment. **E-F.** Early blind weanling climbing up a branch to reach the nesting box .

Figure S2. Behavioral video analyses. A. One frame showing semi-automated identification of an opossum during arena testing using idtracker.ai. **B.** One frame showing behavioral tracking in the gap crossing task using DeepLabCut. 'IR1' and 'IR2' indicate positions of infrared motion sensors mounted on the edge of each platform, tracked using DeepLabCut to aid in gap crossing analyses. **C.** A zoomed in view showing an opossum on the start platform, extending its whiskers towards the goal platform prior to crossing the gap.

Figure S3. **Histological verification of recording sites. A-D.** Myelin stain of a tangential section of the cortex in a standard-reared sighted (sSC) animal (**A**), a standard-reared early blind (sEB) animal (**C**), an enriched sighted (eSC) animal (**E**), and an enriched early blind (eEB) animal (**G**). S1 is readily identified because it is darkly staining compared to adjacent cortical fields. Reconstructions of myeloarchitectural borders drawn from the series of myelin-stained sections in sSC (**B**), sEB (**D**), eSC (**F**) and eEB (**G**) animals. In **B**, **D**, **F** and **G** the location of fluorescent probes is indicated by open circles, and the location of recording sites is indicated by black filled circles. Only recording sites at which whisker-evoked responses of neurons were quantified are shown here. In **A** – **H**, medial (M) and rostral (R) directions are indicated by arrows, and scale bar = 250 μm.

Video S1. A nesting mother opossum navigating the enriched home cage environment. Video shown at 4x speed.

Video S2. Sighted opossum in the arena test. Video shown at 4x speed.

694 **Video S3.** Early blind opossum in the arena test. Video shown at 4x speed.

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Figure 1

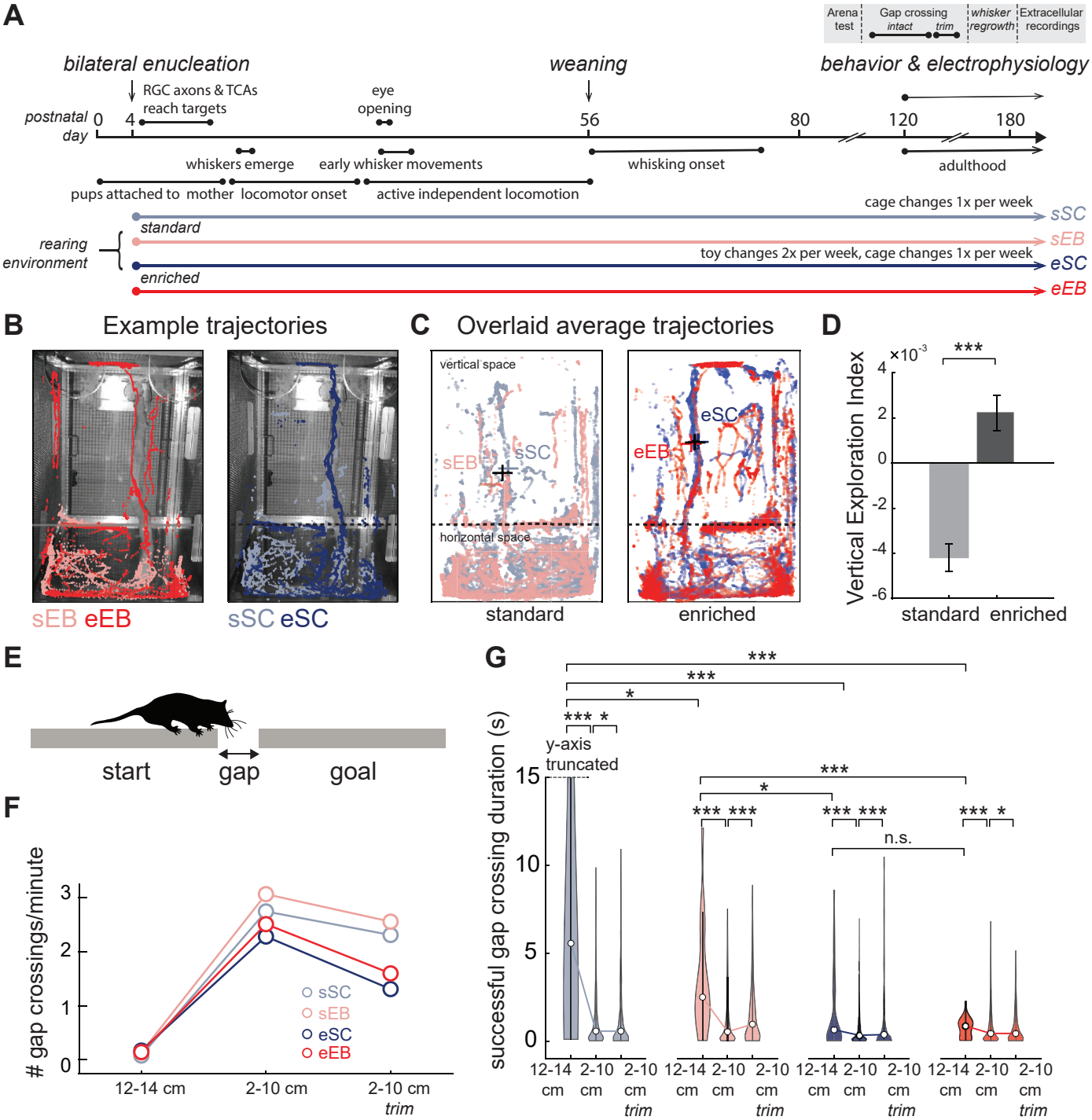
A
Arena test
Gap crossing
intact
trim
whisker regrowth
Extracellular recordings
bilateral enucleation
weaning
behavior & electrophysiology
RGC axons & TCAs reach targets
eye opening
postnatal day
0
4
56
80
120
180
whiskers emerge
early whisker movements
whisking onset
adulthood
pups attached to mother
locomotor onset
active independent locomotion
cage changes 1x per week
sSC
standard
sEB
rearing environment
toy changes 2x per week, cage changes 1x per week
eSC
enriched
eEB
B
Example trajectories
sEB eEB
sSC eSC
C
Overlaid average trajectories
vertical space
horizontal space
sEB
sSC
eSC
eEB
standard
enriched
D
Vertical Exploration Index
×10^-3
***
standard
enriched
E
start
gap
goal
F
gap crossings/minute
sSC
sEB
eSC
eEB
12-14 cm
2-10 cm
2-10 cm trim
G
successful gap crossing duration (s)
y-axis truncated
n.s.
12-14 cm
2-10 cm
2-10 cm trim

Figure 2

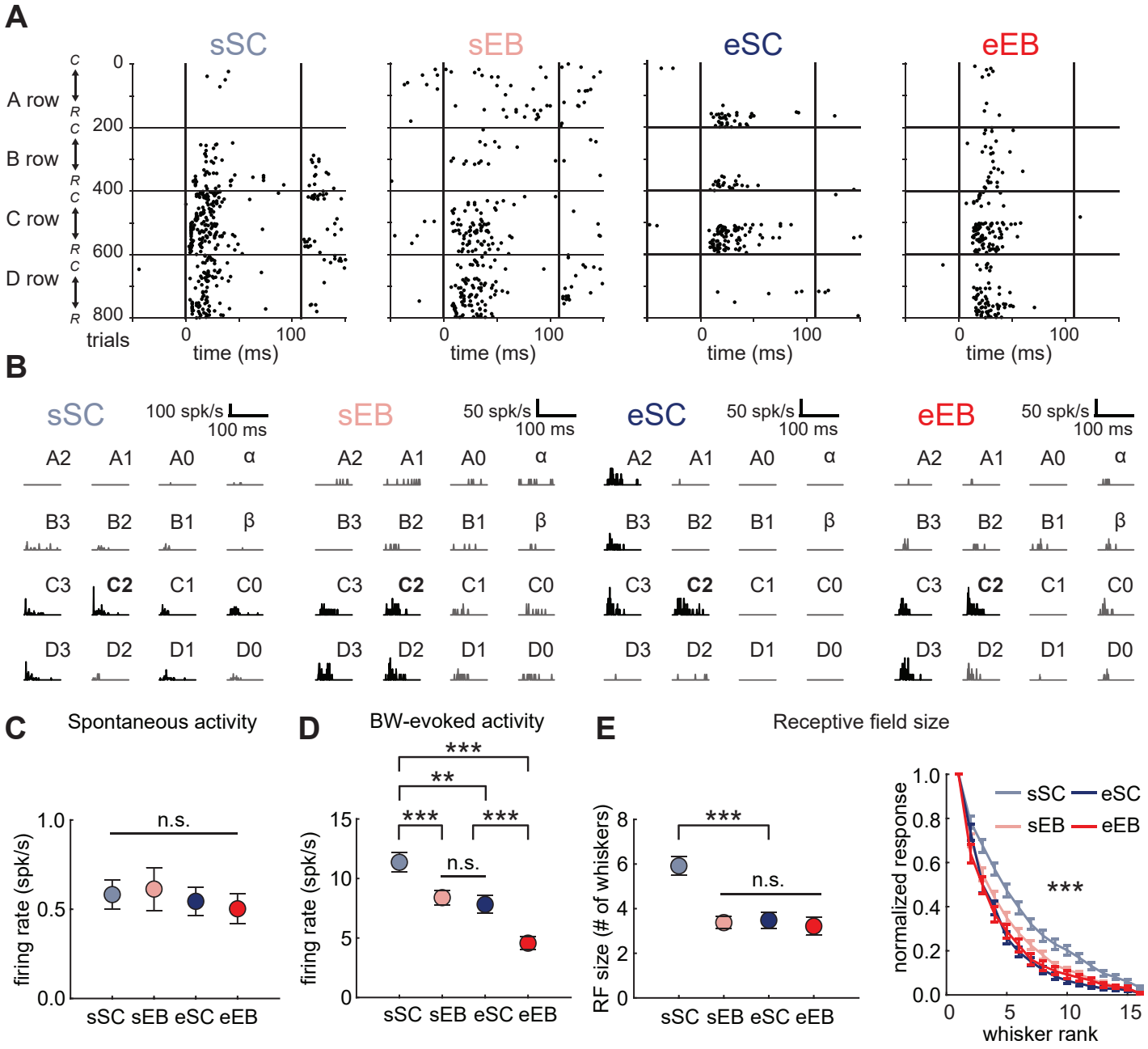
A
sSC
sEB
eSC
eEB
A row
B row
C row
D row
trials
time (ms)
B
100 spk/s
50 spk/s
100 ms
A2 A1 A0 α
B3 B2 B1 β
C3 C2 C1 C0
D3 D2 D1 D0
C
Spontaneous activity
n.s.
firing rate (spk/s)
sSC sEB eSC eEB
D
BW-evoked activity
***
**
n.s.
E
Receptive field size
RF size (# of whiskers)
normalized response
whisker rank

Figure 3

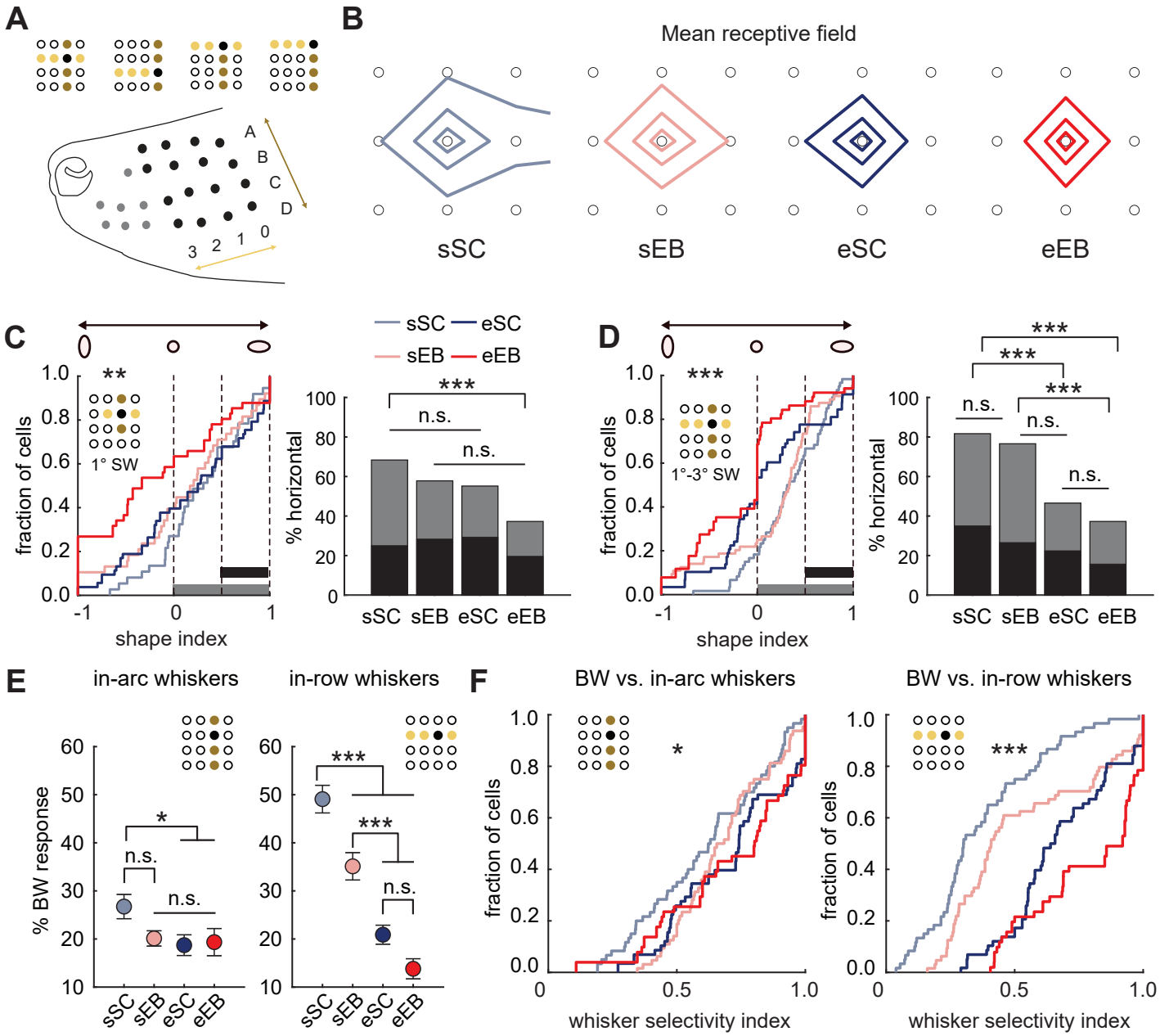
A
B
Mean receptive field
sSC
sEB
eSC
eEB
C
D
fraction of cells
shape index
1° SW
1°-3° SW
% horizontal
n.s.
E
in-arc whiskers
in-row whiskers
% BW response
F
BW vs. in-arc whiskers
BW vs. in-row whiskers
whisker selectivity index

Figure S1

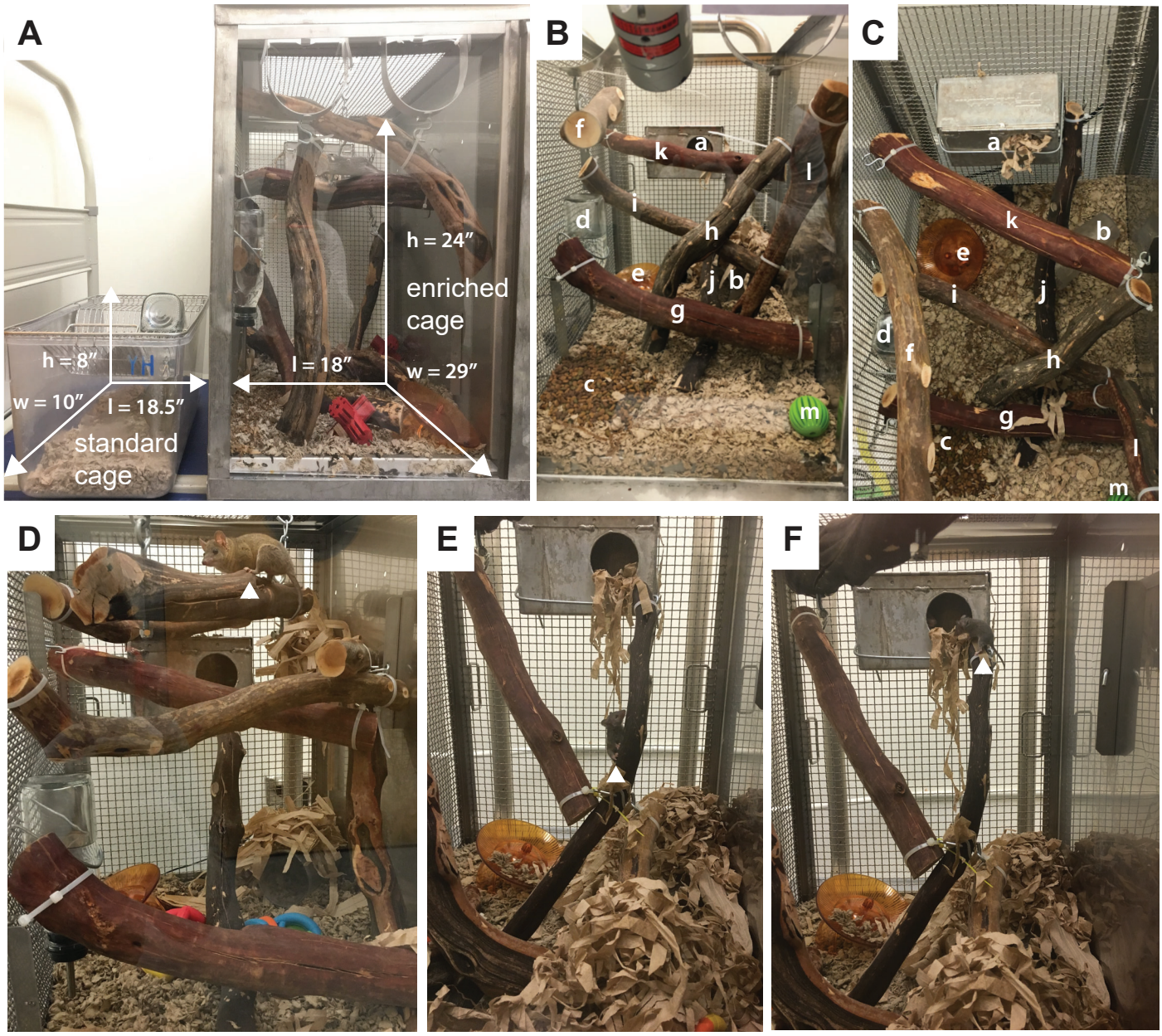
A
h = 8″
w = 10″
l = 18.5″
standard cage
h = 24″
enriched cage
l = 18″
w = 29″
B
a
b
c
d
e
f
g
h
i
j
k
l
m
C
a
b
c
d
e
f
g
h
i
j
k
l
m
D
E
F

Figure S2

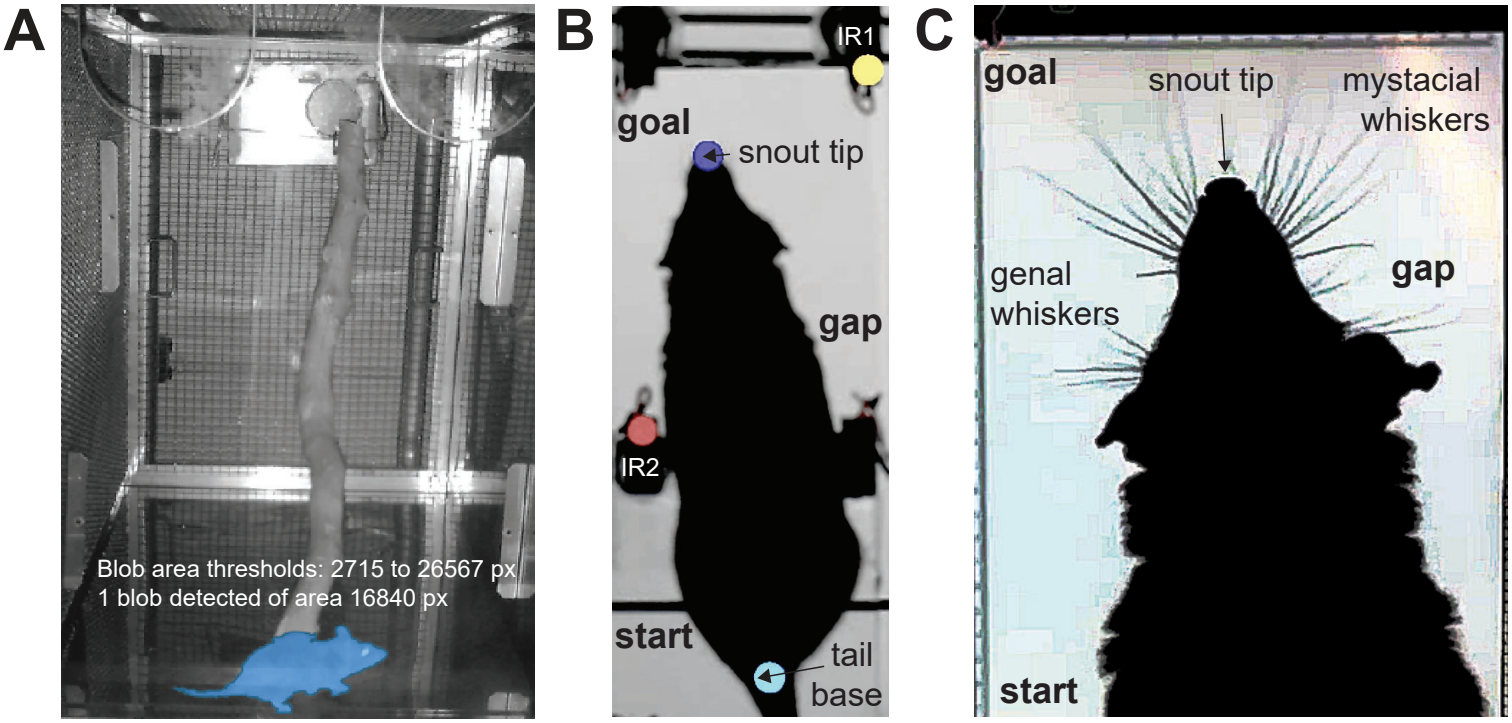
A
Blob area thresholds: 2715 to 26567 px
1 blob detected of area 16840 px
B
IR1
goal
snout tip
gap
IR2
start
tail
base
C
goal
snout tip
mystacial
whiskers
genal
whiskers
gap
start

Figure S3

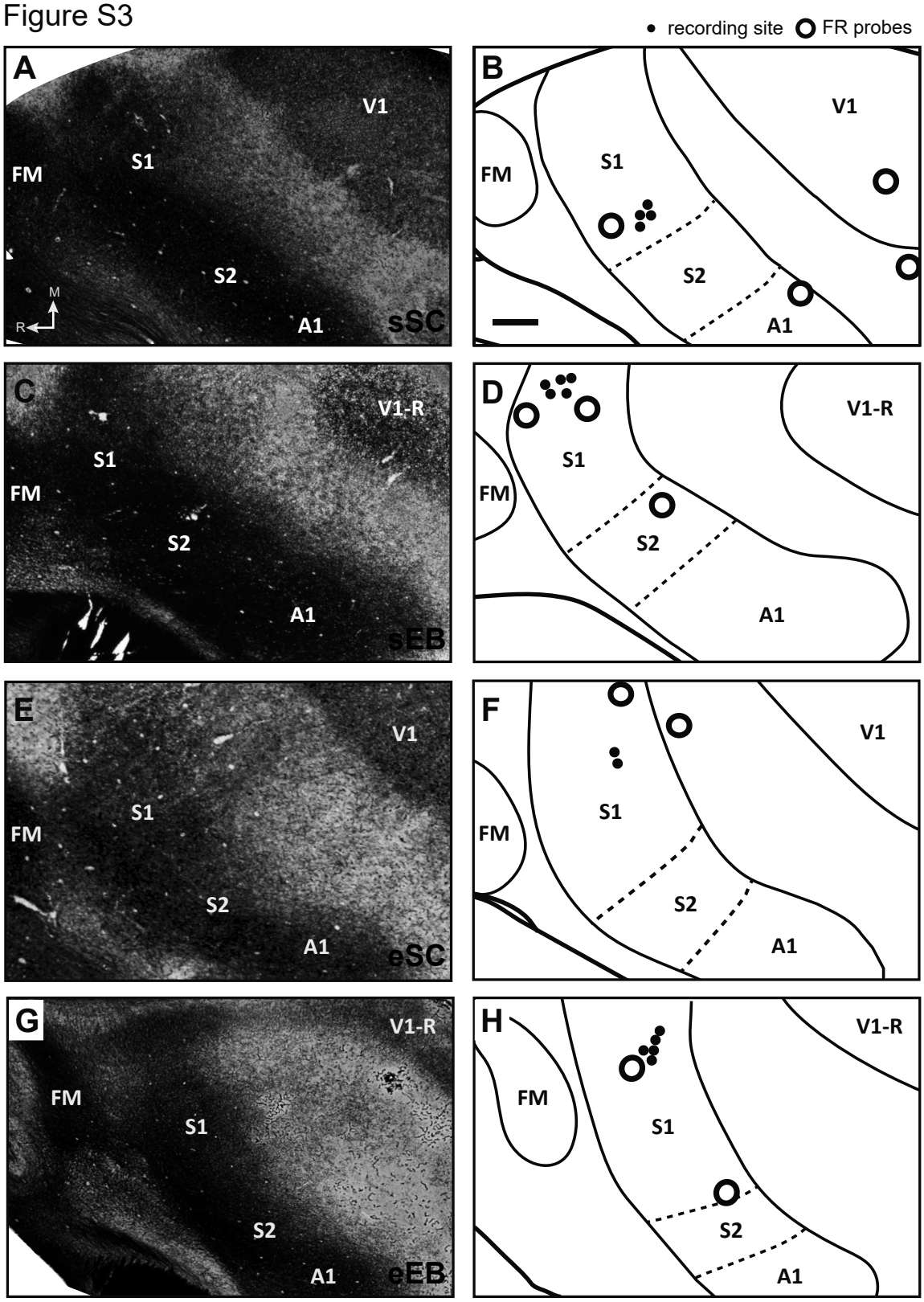
recording site
FR probes
A
V1
S1
FM
S2
M
R
A1
sSC
B
V1
FM
S1
S2
A1
C
V1-R
S1
FM
S2
A1
sEB
D
V1-R
S1
FM
S2
A1
E
V1
S1
FM
S2
A1
eSC
F
V1
S1
FM
S2
A1
G
V1-R
FM
S1
S2
A1
eEB
H
V1-R
FM
S1
S2
A1

Table S1. Experimental Design.

		Visual Condition					
		Sighted (SC)			Early Blind (EB)		
		Sex	ALL	>2 SD	Sex	ALL	>2 SD
Rearing Condition	Standard (s)	5F, 5M	86	60	4F, 5M	88	64
	Enriched (e)	3F, 4M	84	58	3F, 4M	81	51

Breakdown of subject information for each experimental group. Sexes of animals used for electrophysiological recording experiments, total number of neurons recorded in S1 per group (ALL) and number of S1 neurons with significant whisker-evoked responses (>2 SD) are listed.

Table S2. Statistical comparisons for key results

Result/ Figure Panel	Fixed Effect p-value	Pairwise comparisons p-values					
Gap crossing duration **Fig. 1G**	**Test Condition**	**sSC**		**sEB**	**eSC**		**eEB**
(1) large vs. small	2.77e-03	1e-04		1e-04	1e-04		1e-04
(2) intact vs. trim	0.014	0.039		1e-04	1e-04		0.033
	Expt. Group	**sSC vs.sEB**	**sSC vs. eSC**	**sSC vs. eEB**	**sEB vs. eEB**	**sEB vs. eSC**	**eSC vs. eEB**
Gap crossing duration **Fig. 1G** (3) across groups	1.15e-03	0.014	1e-04	2.0e-04	0.012	1e-04	0.066
Spontaneous activity **Fig. 2C**	0.841	-	-	-	-	-	-
Whisker-evoked activity **Fig. 2D**	7.16e-10	1.77e-04	0.005	1.86e-11	1.57e-04	0.550	6.90e-05
RF size: # of whiskers **Fig. 2E**	1.58e-07	9.43e-07	4.29e-06	8.86e-07	0.763	0.832	0.621
Rank-ordered whisker tuning curve **Fig. 2F**	2.68e-17	2.09e-09	5.63e-13	1.74e-15	0.006	0.048	0.532
Shape index: 1st order adjacent whiskers **Fig. 3C**	0.004	0.249	0.445	6.84e-04	0.023	0.628	0.003
% horizontal: 1st order adjacent whiskers **Fig. 3D** Alpha with Bonferroni correction = 0.008	-	0.267	0.185	0.001	0.039	0.855	0.083
Shape index: 1st – 3rd order adjacent whiskers **Fig. 3E**	3.45e-05	0.093	0.013	2.19e-06	0.001	0.384	0.016
% horizontal: 1st – 3rd order adjacent whiskers **Fig. 3F** Alpha with Bonferroni correction = 0.008	-	0.516	9.88e-05	2.00e-06	2.50e-05	7.73e-04	0.340
% BW response: in-arc whiskers **Fig. 3G**	0.042	0.033	0.012	0.024	0.812	0.652	0.848
% BW response: in-row whiskers **Fig. 3G**	8.51e-20	7.10e-05	7.59e-14	1.12e-18	1.25e-08	6.19e-05	0.06
BW vs. in-arc whiskers **Fig. 3H**	0.043	0.08	0.014	0.015	0.419	0.436	0.957
BW vs. in-row whiskers **Fig. 3H**	2.49e-18	2.86e-04	8.18e-12	7.00e-18	1.25e-08	6.19e-05	0.06

Exact p-values for the fixed effect of experimental group and/or test condition in the linear mixed-effects model (see Methods), and pairwise comparisons across experimental groups.