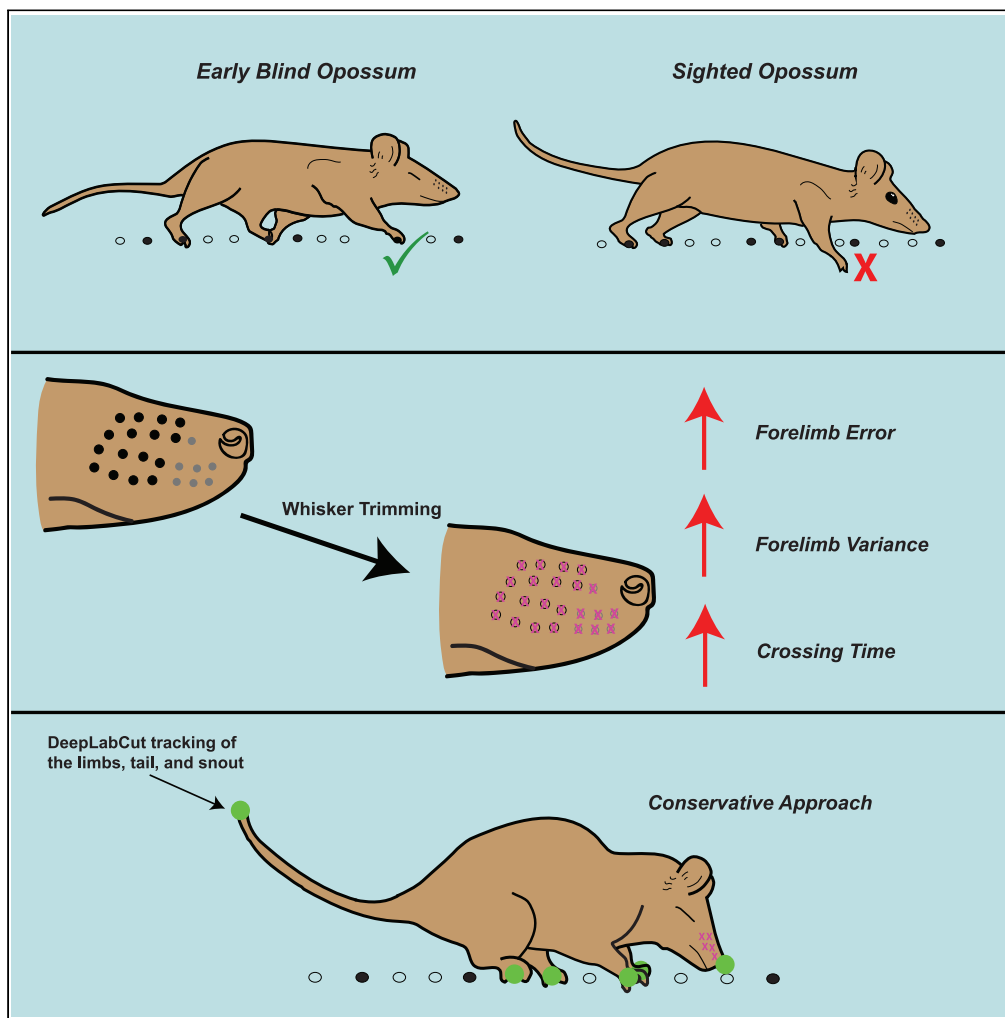


Article

Available Sensory Input Determines Motor Performance and Strategy in Early Blind and Sighted Short-Tailed Opossums



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HIGHLIGHTS

Early blind opossums outperform sighted controls during ladder rung walking

Whisker trimming causes forelimb accuracy deficits in blind and sighted opossums

Whisker trimming, but not the loss of vision, impacts stereotypical movements

Both groups adopt conservative approaches to ladder crossing after whisker trimming

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Article

Available Sensory Input Determines Motor Performance and Strategy in Early Blind and Sighted Short-Tailed Opossums

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SUMMARY

The early loss of vision results in a reorganized neocortex, affecting areas of the brain that process both the spared and lost senses, and leads to heightened abilities on discrimination tasks involving the spared senses. Here, we used performance measures and machine learning algorithms that quantify behavioral strategy to determine if and how early vision loss alters adaptive sensorimotor behavior. We tested opossums on a motor task involving somatosensation and found that early blind animals had increased limb placement accuracy compared with sighted controls, while showing similarities in crossing strategy. However, increased reliance on tactile inputs in early blind animals resulted in greater deficits in limb placement and behavioral flexibility when the whiskers were trimmed. These data show that compensatory cross-modal plasticity extends beyond sensory discrimination tasks to motor tasks involving the spared senses and highlights the importance of whiskers in guiding forelimb control.

INTRODUCTION

Navigation through complex space requires a tight coupling between incoming sensory input and motor output (Abbruzzese and Berardelli, 2003; Ferezou et al., 2007). The ability of an animal to make precise, sensory-guided movements while actively exploring the environment depends on the sensory information available, the structure of the peripheral epithelia, the morphology of the body, and the neural circuits involved in integrating sensory information from different modalities. Because evolution has produced species who rely on some senses over others, it is important to look at animals that have different body morphologies and that rely on different combinations of sensory input to appreciate general principles of nervous system structure and function that allow mammals to adapt movement strategies in a constantly changing environment. For example, cats and humans use vision to guide complex locomotion (Drew and Marigold, 2015; McVea et al., 2009), bats rely on echolocation to navigate three-dimensional space (Moss and Surlykke, 2010), and many rodents employ tactile inputs relayed through the whiskers to navigate terrestrial and arboreal habitats (Grant et al., 2009; Mitchinson et al., 2011). Although different species often rely heavily on one sense, there is accumulating evidence that the developing neocortex is not as strictly constrained by its evolutionary history as previously thought, since the loss of sensory input early in development leads to a massive restructuring of the neocortex based on the remaining sensory inputs (Bell et al., 2019; Cecchetti et al., 2016; Kupers and Ptito, 2014; Renier et al., 2014).

Studies of early vision loss in animal models and humans demonstrate that the neocortex is capable of remarkable functional and anatomical plasticity, and this plasticity appears to support important sensory-mediated behaviors. For example, if visual input is lost in developing mice, rats, and hamsters, neurons in visual cortex (V1) respond to deflections of the whiskers and to auditory stimuli such as tones and clicks (Izraeli et al., 2002; Piche et al., 2007; Toldi et al., 1990). Cortico-cortical connections are also altered by the early loss of vision, as rats enucleated at birth show increased variability and divergence of intra- and interhemispheric connections between V1 and extrastriate cortex (Bock and Olavarria, 2011; Laing et al., 2012). In mice, the early loss of vision alters cortico-cortical connections prior to would-be eye opening, such that projections from S1 to V1 are more broadly distributed in blind mice compared with sighted controls (Dye et al., 2012; Kozanian et al., 2015).

Work from our laboratory in short-tailed opossums (*Monodelphis domestica*) has demonstrated that removing all visual input prior to the onset of spontaneous activity from the retina results in massive

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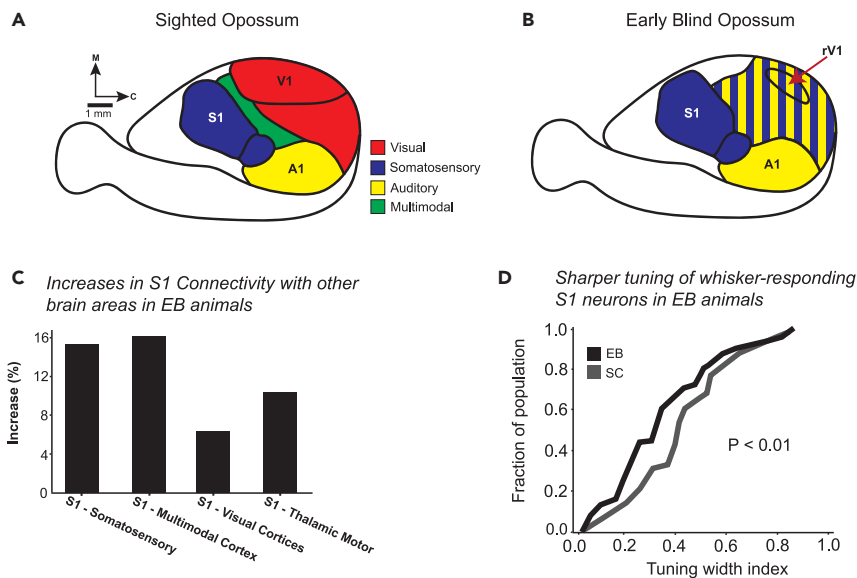


Figure 1. The Early Loss of Vision Results in Drastic Changes to the Neocortex

(A) Previous experiments from our laboratory show that a large portion of the *Monodelphis* neocortex responds to visual (red) and somatosensory (blue) cues.

(B) In P4 early blind animals, visual cortex is reorganized by the somatosensory and auditory system (rV1).

(C) This is accompanied by an increase in connectivity between S1 and other somatosensory cortices (S1-S2/SR/SC), as well as between S1 and multimodal cortex (S1-MM), visual cortices (S1-V1/CT), and thalamic motor nuclei (S1-VL/VA).

(D) Within somatosensory cortex, whisker-responding neurons are more sharply tuned in EB (black) compared with SC animals (gray). Abbreviations: S1, primary somatosensory cortex; S2, secondary somatosensory cortex; SC, caudal somatosensory area; SR, rostral somatosensory area; MM, multimodal cortex; V1, primary visual cortex; CT caudal temporal area; VL, ventral lateral; VA, ventral anterior nuclei of the thalamus. Figures adapted from Dooley and Krubitzer, 2019; Karlen et al., 2006, and Ramamurthy and Krubitzer (2018).

cross-modal changes in the functional organization and connections of what would have been visual cortex (Kahn and Krubitzer, 2002; Karlen and Krubitzer, 2009). Similar to those of rodents, neurons in the reorganized visual cortex of early blind (EB) opossums respond to stimulation of the face and whiskers, and to broadband auditory clicks (Figures 1A and 1B) (Karlen et al., 2006). The spared sensory systems are also affected. There are alterations in cortical and subcortical connections of primary somatosensory cortex (S1) (Figure 1C) (Dooley and Krubitzer, 2019; Karlen et al., 2006) and a spatial sharpening of receptive fields and improved population decoding for whisker-stimulus position for neurons in S1 (Figure 1D) (Ramamurthy and Krubitzer, 2018), indicating that individual neurons in S1 in EB opossums have better discriminability.

Given the overwhelming evidence from our laboratory and others that early vision loss results in drastic cross-modal changes to sensory and association cortical areas, it is surprising that only a few studies have investigated the behavioral outcomes that result from these neural changes, and none have quantified how kinematics, strategy, and performance during navigation are altered in tandem (Rauschecker, 1995; Renier et al., 2014). One study in rats found that early blind animals perform a maze-running task more quickly and have alterations in the receptive-field size of neurons in barrel cortex (Toldi et al., 1994), whereas a finger-maze study in blind humans found no difference in performance compared with sighted participants (Gagnon et al., 2012). Other studies have found that blind individuals can create mental maps of space more efficiently than sighted controls (for review see Schinazi et al., 2016). This limited body of research leaves the sensorimotor behavioral correlates to cross-modal changes in the brain largely unknown.

The goal of the present investigation was to assess differences in both performance and strategy between early blind and sighted opossums on a naturalistic sensorimotor task that requires coordination of the limbs and postural control of the body and head to navigate across a relatively complex substrate (ladder rung

apparatus). We sought to determine the extent to which animals rely on the remaining available sensory information by removing sensory input from the whiskers. To accomplish this, we utilized recently developed, marker-less tracking algorithms to analyze performance, locomotor patterns, and movement strategies captured on video in short-tailed opossums bilaterally enucleated at postnatal day 4, prior to the onset of spontaneous retinal activity and the formation of thalamic and cortical visual pathways. As *Monodelphis* is among mammals that use the whiskers for active sensing (whisking), we used a sensorimotor task known to involve somatosensation: the variable ladder-rung walking task (Metz and Whishaw, 2009; Mitchinson et al., 2011). This task is sensitive to minute differences in motor control of the limbs and has been used to assess motor deficits in peripheral and neurologic disease models (Antonow-Schlorke et al., 2013; Metz and Whishaw, 2002; Schonfeld et al., 2017).

RESULTS

In the following results, we compared both performance and movement strategy in early blind (EB) and sighted control (SC) animals while performing the ladder rung task. Statistics are reported as Adjusted R-Squared (R^2_{adj}), and the F-Statistic is presented with degrees of freedom of the model, F(N). Delta (Δ) indicates the difference between pre whisker-trim and post whisker-trim means within early blind (ΔEB) and sighted opossums (ΔSC).

Early Blind Opossums Outperform Sighted Controls in Variable Ladder Rung Walking but Show Similarities in Crossing Strategy

To determine differences in ladder rung walking performance between early blind and sighted opossums, foot faults on nine variable ladder rung patterns were scored by two independent observers. Using a linear model testing for interactions between experimental group and lighting condition, we found a significant main effect for sightedness on total error, with early blind opossums committing fewer errors than sighted controls ($R^2_{adj} = 0.32$, $F(8) = 6.62$, $p = 0.007$) (Figure 2A). Under the same model, we found no significant effect of lighting ($p = 0.718$), with animals in both experimental groups performing similarly in the light and dark (Figure S1C). Furthermore, there was no effect of biological sex on performance ($p = 0.086$). We found no differences in performance across different variable patterns or in the order in which rung patterns were completed (light or dark first) ($R^2_{adj} = 0.09$, $F(3) = 8.00$, $p = 0.367$, $p = 0.207$) (Figure S1A). Because there was no significant effect for lighting, a reduced model was used to test the individual contributions of forelimb and hindlimb error to total error rate.

Additionally, as opossums completed at least three standard patterns on all 7 days prior to walking variable patterns, we analyzed performance on standard patterns across testing days. Group differences were significant on day 1 (habituation day, no variable patterns), with EB opossums performing worse than SC opossums ($p = 0.01$). However, on all testing days (days 2–7), there was no significant difference between EB and SC performance on standard patterns ($p_{2-7} > 0.40$). However, whisker trimming did significantly decrease performance on standard patterns ($p < 0.001$).

On variable patterns, early blind opossums committed significantly less forelimb errors than sighted animals ($R^2_{adj} = 0.32$, $F(4) = 11.81$, $p = 0.004$) (Figure 2B). Additionally, early blind opossums committed significantly less hindlimb errors than sighted animals ($R^2_{adj} = 0.10$, $F(4) = 3.71$, $p = 0.008$) (Figure 2B). Thus, in the absence of vision, early blind opossums outperformed their sighted counterparts, making significantly fewer errors during rung walking whether fore- and hindlimbs were considered separately or together.

To investigate the crossing strategies and kinematics that may underlie differences in performance, we quantified crossing time and nose tapping behavior and used DeepLabCut marker-less tracking in a randomly chosen subset of animals ($n = 8$) to track the position of the snout, limbs, and tail as animals crossed the ladder (Figure S4 and Video S1). We found no significant effect of crossing time on performance ($R^2_{adj} = 0.35$, $F(4) = 10.71$, $p = 0.23$) and no significant difference in crossing time between early blind (7.85 ± 0.72 s) and sighted animals (6.64 ± 0.9 s) ($R^2_{adj} = 0.24$, $F(4) = 7.67$, $p = 0.27$) (Figure 2C). There was also no significant difference in the average frequency of nose taps per-trial between blind and sighted animals ($R^2_{adj} = 0.34$, $F(4) = 7.57$, $p = 0.63$) (Figure 2D) or the average height at which animals held their snout during correct placements ($R^2_{adj} = 0.60$, $F(4) = 6.46$, $p = 0.84$) (Figure 2E). These results show that animals in both groups take similar amounts of time to cross the ladder and implement some of the same strategies while doing so, but early blind animals show increased performance.

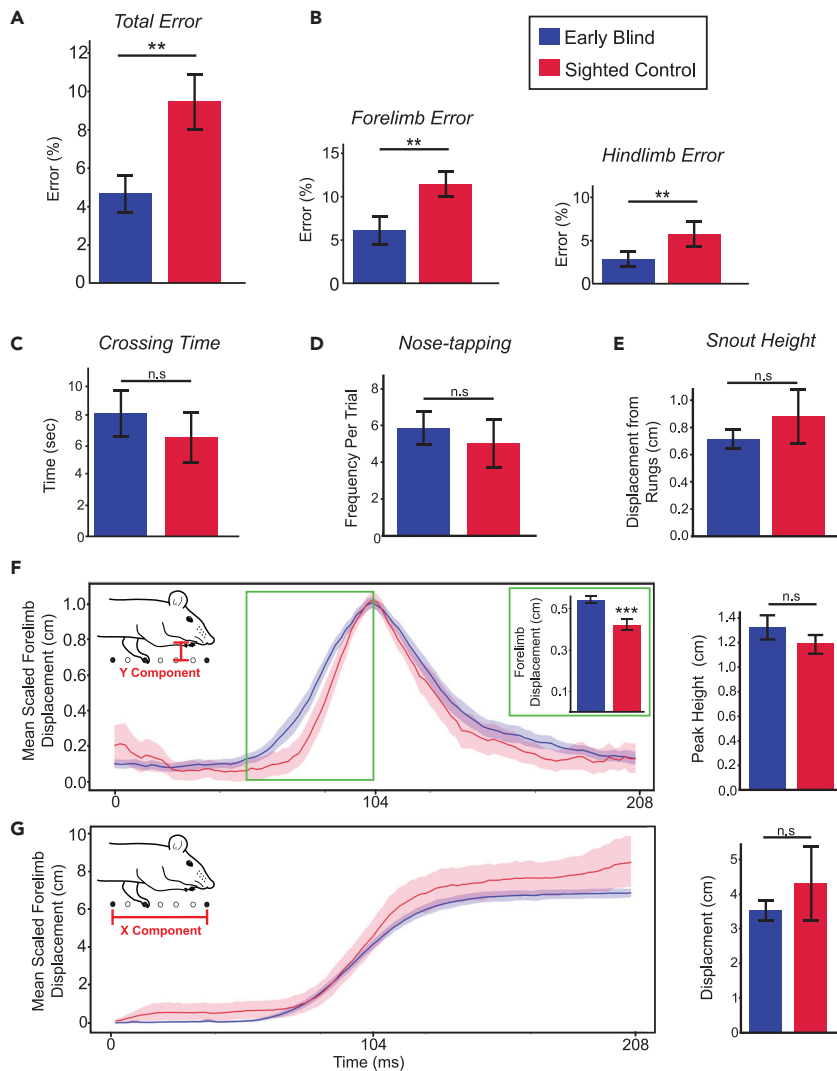


Figure 2. Early Blind Opossums Outperform Sighted Controls in Variable Ladder Rung Walking but Show Similarities in Crossing Strategy

(A) Bar graphs showing that early blind opossums commit significantly less error than sighted opossums ($p = 0.007$). (B) Bar graphs of average forelimb and hindlimb error. On average, early blind animals commit significantly less forelimb ($p = 0.004$) and hindlimb ($p = 0.008$) error than sighted controls. (C–E) (C) We found no significant difference in crossing time ($p = 0.27$), (D) average number of nose taps per trial ($p = 0.626$), or (E) snout height during correct forelimb placements between conditions ($p = 0.806$). (F) Line graphs depicting the average y component (step height) of forelimb trajectories during correct placements ($n = 99$ EB; 56 SC) of eight animals ($n = 4$ EB; $n = 4$ SC), scaled by average peak height per animal (inset image, left panel). The green square and inset bar graph denotes the region of non-overlap of 95% confidence intervals, where early blind animals are quicker to lift their right forelimb off of a rung ($p < 0.001$). Bar graph shows quantification of average y-component forelimb trajectory as average peak height (right). Note: bar graph does not include scaling to illustrate there is no statistical difference in peak height between conditions prior to scaling ($p = 0.267$). (G) Line graphs depicting forelimb displacement in the x-direction (stride length). Quantification of average displacement shows no difference in the average x-component of forelimb movement between early blind and sighted opossums ($p = 0.405$) (right). All statistical tests were calculated using linear models (see [Transparent Methods: reduced model](#)). (* $p < .05$, ** $p < .01$, *** $p < .001$, n.s. = $p > .05$).

Bar graphs are presented as between-group averages. Error bars are presented as 95% bootstrapped confidence intervals. See also [Figure S1](#).

Next, we quantified average forelimb trajectories during correct placements in both groups ($n = 155$ forelimb motions). Average peak height did not differ between groups ($R^2_{\text{adj}} = 0.24$, $F(4) = 7.67$, $p = 0.27$) (Figure 2F). However, during forelimb motion, early blind animals were relatively quicker to lift their forelimb compared with sighted control animals ($R^2_{\text{adj}} = 0.72$, $F(4) = 1646.0$, $p < 0.001$) (Figure 2F; green rectangle). This was quantified as the average height of the limb during the second quartile of a forelimb motion (52–104 ms). Surprisingly, average forelimb peak height significantly predicted average forelimb error, with higher peaks indicating better performance ($R^2_{\text{adj}} = 0.78$, $F(4) = 13.06$, $p = 0.028$). Although one might expect larger movements to be correlated with more errors, peak height may be an indicator of an animal's confidence in its estimation of where the next rung is located. In the X-direction (step length), both early blind and sighted opossums exhibit similar forelimb kinematics (i.e., similar step lengths), possibly constrained by the finite spacing of the rungs (Figure 2G). Thus, apart from differences in when the forelimb was lifted off of a rung, there was no overall difference in the behavioral strategy shown by the metrics that we used. However, it should be noted that differences in the kinematics of other body parts that we did not quantify, particularly in how the position of the whiskers relates to the position of the forelimbs, may also contribute to differences in performance.

Whisker Trimming Causes Deficits in Performance in Forelimb but Not Hindlimb Placement; Performance Deficits and Limb Trajectories Are Altered to Greater Extents in Early Blind Animals

Work from our laboratory has recently shown that neurons in the whisker representation of S1 have smaller receptive fields and increased selectivity (i.e., greater discriminability) (Ramamurthy and Krubitzer, 2018). Thus, we examined the role of the whiskers during variable ladder rung walking. To accomplish this, we trimmed the mystacial, submandibular, and genal vibrissae and re-tested animals on variable patterns over the course of two additional testing days. We found a significant main effect for whisker trimming on overall performance in early blind and sighted animals ($R^2_{\text{adj}} = 0.31$, $F(4) = 11.62$, $p < 0.001$, $\Delta\text{EB} = +5.57\%$ error, $\Delta\text{SC} = +2.90\%$ error) (Figures 3A and S3A). Although absolute increases in error were ~6% and 3% for EB and SC opossums, respectively, relative increases in error were 50% and 33% (comparing pre- and post-trim means), showing the extreme detriment of whisker trimming to rung walking performance. Moreover, increases in error due to whisker trimming are driven by increases in forelimb error ($R^2_{\text{adj}} = 0.32$, $F(4) = 11.81$, $p < 0.001$, $\Delta\text{EB} = +8.81\%$ error, $\Delta\text{SC} = +5.92\%$ error) but not hindlimb error ($R^2_{\text{adj}} = 0.10$, $F(4) = 3.71$, $p = 0.40$, $\Delta\text{EB} = +1.09\%$ error, $\Delta\text{SC} = +0.65\%$ error) (Figure 3B). Whisker trimming caused a 2-fold increase in total error in early blind opossums, increasing their error rate to similar levels to those observed in sighted animals with whiskers, highlighting their heavy reliance on this spared sensory system. We found that, overall, average forelimb error significantly predicted average hindlimb error ($R^2_{\text{adj}} = 0.32$, $p < 0.001$ for animals with whiskers, and $R^2_{\text{adj}} = 0.10$, $p = 0.03$ for whisker trimmed animals) (Figure 3C), illustrating the relationship between forelimb and hindlimb movement in precision quadrupedal locomotion, as animals that were accurate forelimb placers were also accurate hindlimb placers.

Next, to assess how the absence of tactile sensory information provided by the whiskers altered limb placement, we compared forelimb and hindlimb trajectories during correct forelimb placements before and after whisker trimming using data derived from DeepLabCut marker-less tracking. We found that whisker trimming differentially altered the trajectories of forelimb motion in early blind and sighted animals, such that sighted animals exhibited little reduction in peak height, whereas whisker-trimmed early blind animals show a significant reduction in forelimb peak height during correct placement ($R^2_{\text{adj}} = 0.40$, $F(4) = 3.46$, $p = 0.02$, $\Delta\text{EB} = -0.27$ cm, $\Delta\text{SC} = -0.1$ cm) (Figures 3D, 3E, and S1D). The 2.5-fold differential reduction in average forelimb peak height between groups demonstrates the greater effect of whisker trimming on early blind opossums' sensorimotor coordination. The X-component (step length) of forelimb trajectories during correct placements was also altered due to whisker trimming, resulting in shorter steps for both groups ($\Delta\text{EB} = -0.8$ cm, $\Delta\text{SC} = -1.69$ cm) (Figures 3F and 3G). Likewise, hindlimb trajectories in both groups became shallower after whisker trimming (Figures 3H and 3I). Interestingly, the average reduction in hindlimb peak height matched the reduction in forelimb peak height in EB opossums exactly ($\Delta\text{EB} = -0.27$ cm). Again, a less extreme reduction in hindlimb peak height was observed in sighted controls after whisker trimming ($\Delta\text{SC} = -0.23$ cm) (Figure S1D). Together, these data show the importance of whiskers for forelimb placement and, furthermore, the importance of forelimb placement in guiding hindlimb placement. In sum, removing a heavily relied upon sense, especially in early blind animals, results in changes to performance and locomotor trajectories.

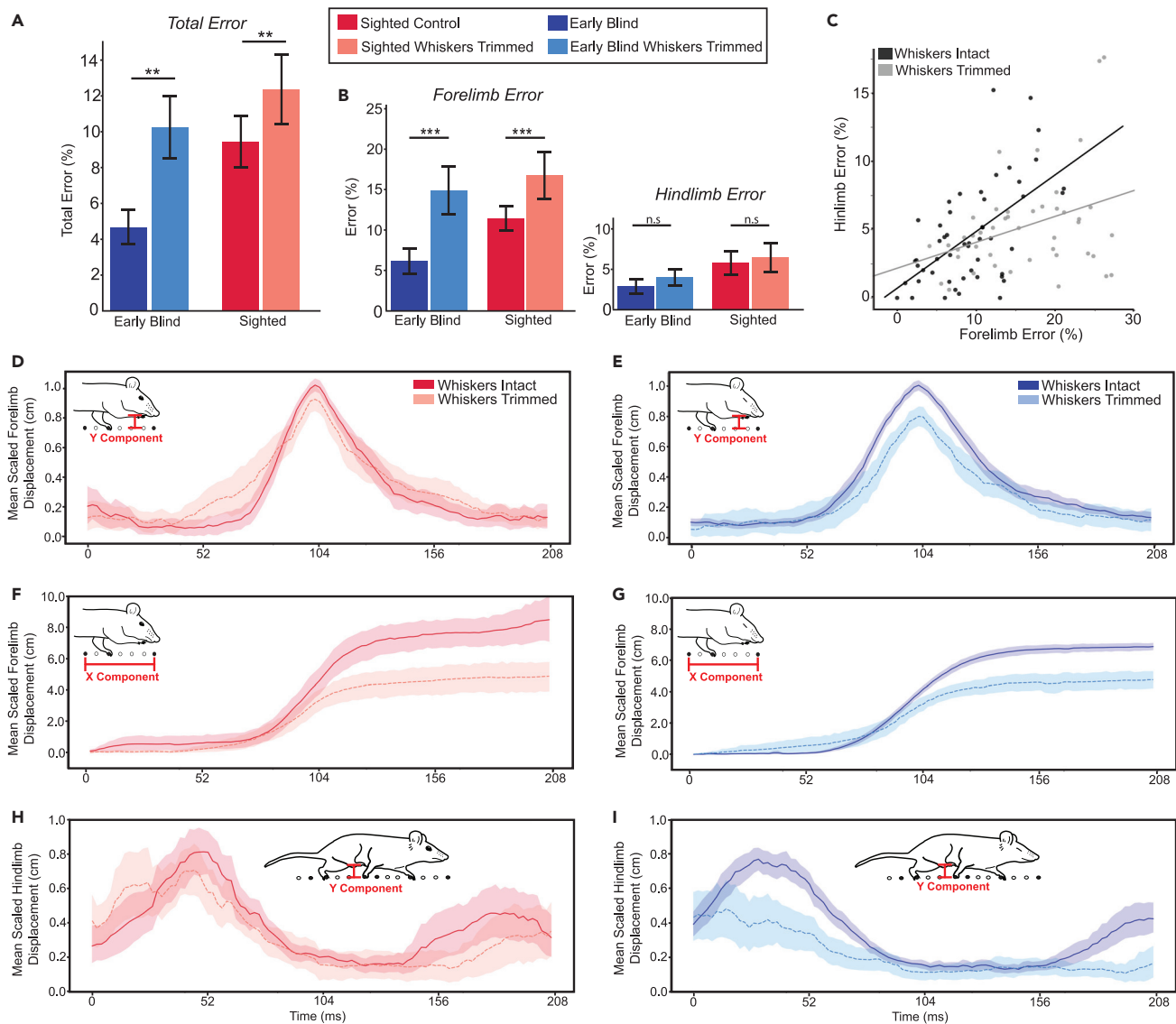


Figure 3. Whisker Trimming Causes Deficits in Performance in Forelimb but Not Hindlimb Placement

Performance deficits and limb trajectories are altered to greater extents in early blind animals.

(A) Bar graph showing average total error before and after whisker trimming in early blind (blue) and sighted (red) opossums. Whisker-trimmed averages are represented by lighter shades (early blind whisker trimmed: light blue; sighted whisker trimmed: light red). A main effect was found for the presence of whiskers on performance ($p = 0.004$), with whisker trimming leading to increases in error.

(B) Forelimb (left) and hindlimb (right) error before and after whisker trimming. Collapsing across lighting condition, the reduced model shows a significant main effect for increases in forelimb ($p < 0.001$) but not hindlimb ($p = 0.397$) error due to whisker trimming. (C) Scatterplot with fit lines showing that by animal, average forelimb error significantly predicts average hindlimb error in the presence ($p < 0.001$) or absence ($p = 0.03$) of whiskers.

(D and E) Line graphs depict average y-component forelimb trajectories before (solid lines) and after whisker trimming (dashed lines) in sighted (D) and early blind opossums (E). Whisker trimming reduces step height to a significant extent ($p = 0.019$).

(F and G) Line graphs depicting forelimb displacement in the x-direction (stride) before and after whisker trimming. Whisker trimming reduces step width in both sighted (F) and early blind opossums (G).

(H and I) Line graphs depict average y-component hindlimb trajectories before and after whisker trimming. Whisker trimming results in shallow hindlimb movements for sighted (H) and blind (I) animals. Overall, trajectories of the limbs are altered to a greater extent in early blind opossums. Line graphs are presented as average trajectories with bootstrapped 95% confidence intervals.

Bar graphs are presented as means with error presented as bootstrapped 95% confidence intervals. Where p values are reported, statistical tests were calculated using linear models (see [Transparent Methods](#): reduced model). ($*p < .05$, $**p < .01$, $***p < .001$, $n.s = p > .05$). See also [Figure S1](#).

Whisker Trimming Alters Forelimb Dynamics by Causing Variable Motions and Different Movement Types

Since whisker trimming resulted in changes to forelimb trajectories, we sought to determine the underlying sources of these changes. Observation from video and trajectory plots indicates that variance in forelimb movements may increase after whisker trimming. Therefore, we assessed the variance of the Y-Component (step height) of forelimb trajectories between early blind and sighted opossums, with and without whiskers. As expected, the lowest variance in all groups was at the beginning, peak, and end of a forelimb movement (Figure 4A). Although this is most likely due to the fact that peaks were aligned for analysis and that ladder rungs were at fixed intervals, restrictions on the musculoskeletal system and muscle synergies also contribute to highly stereotyped movements during locomotion. Variance of forelimb movements was not correlated with the number of strikes analyzed ($R^2_{\text{adj}} = 0.01$, $F(1) = 1.12$, $p = 0.31$). However, by animal, higher variance of correct forelimb placements was found to significantly predict average hindlimb error, again indicating that animals may be less certain of an upcoming rung's position ($R^2_{\text{adj}} = 0.30$, $F(1) = 7.47$, $p = 0.02$).

We then binned our analyses into a pre-peak retraction (frames 20–50) and a post-peak extension (frames 50–80) phase. We excluded the first and last 20 frames of motion from this analysis to ensure frames where the forelimb was potentially resting on a rung were excluded. The retraction phase is noted to be the swing portion of the forelimb movement (Jacobs et al., 2014). This part of the movement begins on a ladder rung. The elbow then moves from full extension to full flexion at the peak of the motion. The extension phase is noted to be the swing-to-stance portion of the limb movement, where the elbow goes from flexion to extension, ending with the forepaw being placed on an upcoming rung. Although there was no difference in the overall variance between early blind ($0.063 \pm 0.007\text{cm}$) and sighted ($0.060 \pm 0.005\text{cm}$) opossums ($R^2_{\text{adj}} = 0.22$, $F(3) = 11.51$, $p = 0.23$), whisker trimming significantly increased variance in both groups during the retraction phase of a forelimb movement ($R^2_{\text{adj}} = 0.22$, $F(3) = 11.51$, $p < 0.001$, $\Delta\text{EB} = +0.046$, $\Delta\text{SC} = +0.036$) (Figure 4B). Conversely, whisker trimming did not significantly affect variance in the extension (placement) phase of forelimb movement ($R^2_{\text{adj}} = 0.042$, $F(3) = 2.69$, $p = 0.21$, $\Delta\text{EB} = -0.011$, $\Delta\text{SC} = -0.015$) (Figures 4A–4D), showing that whiskers are critical for quick and accurate detection of future forelimb placement locations but that the guidance of the motion is not affected once the opossum has already detected a rung.

Next, to characterize stereotypical movement types between groups, we used K-Means clustering to classify forelimb step height waveforms into stereotypical movements. *A priori*, we sorted waveforms by sightedness and whisker presence to detect differences within groups and used the elbow point method and highest silhouette coefficient (SCoef) to determine the number of clusters used for each experimental condition. For sighted animals, both methods converged on three clusters ($\text{SCoef}_3 = 0.24$) (Figures 4E and S2). For early blind animals, the elbow method estimated three clusters (Figure S2), whereas the silhouette method estimated two ($\text{SCoef}_2 = 0.23$, $\text{SCoef}_3 = 0.21$). Thus, both two and three clusters were considered for analysis. Interestingly, forelimb movements of both early blind and sighted animals clustered into similar movement types regardless of whether they were clustered into two or three stereotypical movements (Figure S2). The only observed difference was in the retraction phase of a single movement type of early blind animals (Figure 4E). For both early blind and sighted opossums, the extension phase involved either a quick, medium, or slow placement trajectory.

In whisker-trimmed animals, forelimb movements clustered definitively into two similar types regardless of blind or sighted condition: one movement where the forelimb was lifted off the rung immediately, but took a slow linear trajectory to its peak height, and one quick nonlinear movement with a shallow peak (EB: $\text{SCoef}_2 = 0.23$, $\text{SCoef}_3 = 0.19$) (SC: $\text{SCoef}_2 = 0.31$, $\text{SCoef}_3 = 0.17$) (Figure 4E). Thus, stereotypical movement types were not influenced by the presence or absence of vision but instead by the presence or absence of whiskers. Additionally, movements were most notably changed in the retraction phase, showing increased variability and altered waveform shape, illustrating the importance of whiskers in detecting the upcoming rung.

Opossums Adapt to Whisker Trimming by Altering Body Posture and Strategy

Finally, to examine the alternate strategies used by opossums after whisker trimming, we quantified aspects of body posture during correct forelimb placements. First, we quantified the distance in the X direction (stride length) between the right forelimb and right hindlimb, as well as between the right forelimb and

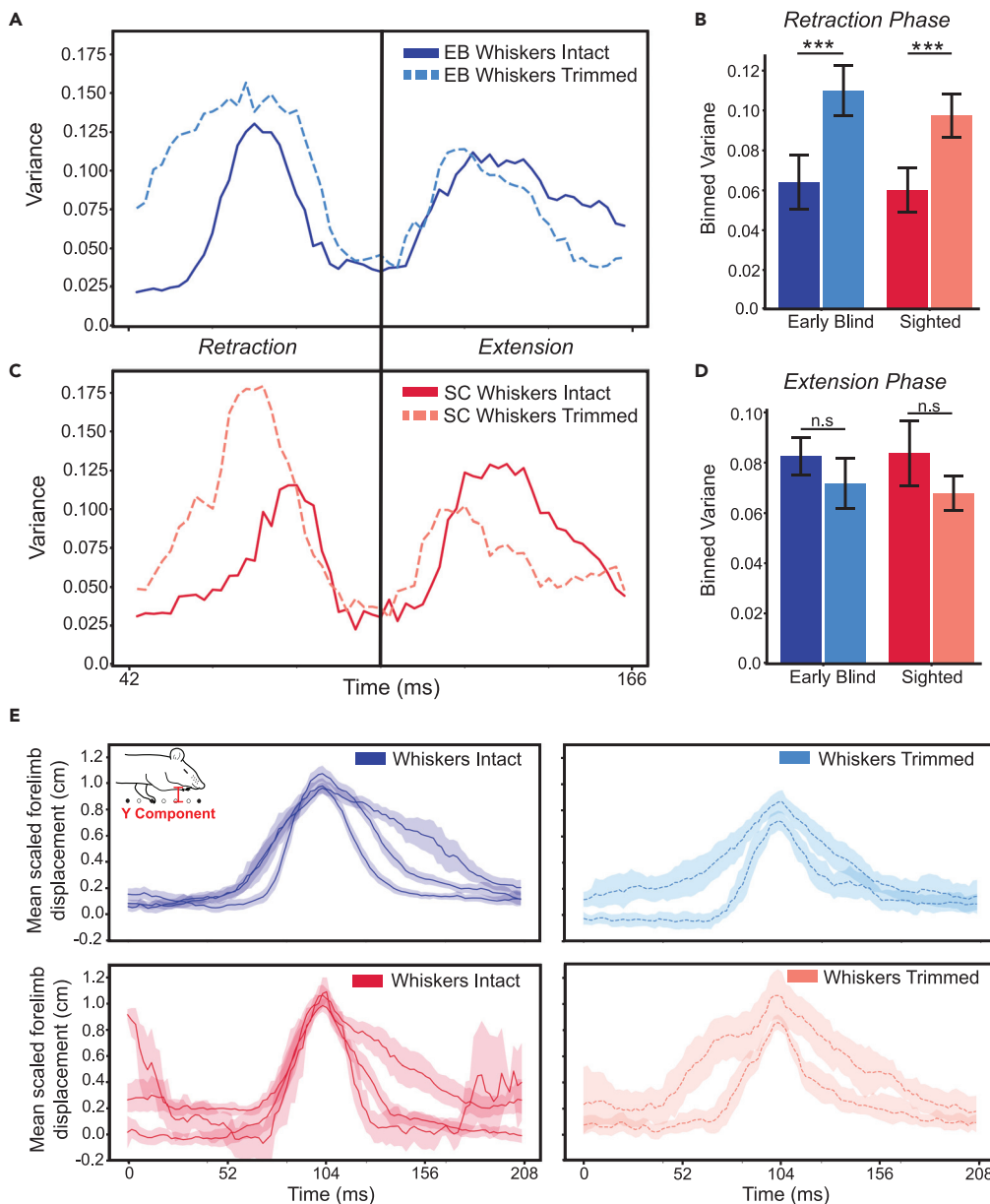


Figure 4. Whisker Trimming Results in More Variable Forelimb Trajectories and Different Types of Stereotypical Movements in Both Early Blind and Sighted Animals

(A and C) Line graphs with bootstrapped 95% confidence intervals of the average variance of y component forelimb trajectories across all motions ($n = 155$) before (solid lines) and after whisker trimming (dashed lines) in early blind (A) and sighted (C) opossums. The vertical black line denotes the separation between retraction and extension phases.

(B and D) Bar graphs show mean binned variance with 95% confidence intervals during retraction (B) and extension (D) phases. Binned variance is the average variance of the entire retraction or extension phase. Whisker trimming significantly increases variance during the retraction ($p < 0.001$) but not extension ($p = 0.21$) phase of forelimb movement (see [Transparent Methods](#): reduced model).

(E) Stereotyped movements of early blind and sighted opossums before and after whisker trimming provided by K-means clustering presented as average line graphs with 95% confidence intervals. The elbow point and silhouette coefficient were used to determine the number of clusters (see [Transparent Methods](#)). Stereotypical movements are similar in early blind (top left) and sighted (bottom left) opossums. Whisker trimming alters stereotypical movements in similar ways in early blind (top right) and sighted (bottom right) opossums.

Where p values are reported: * $p < .05$, ** $p < .01$, *** $p < .001$, n.s. = $p > .05$. See also [Figure S2](#).

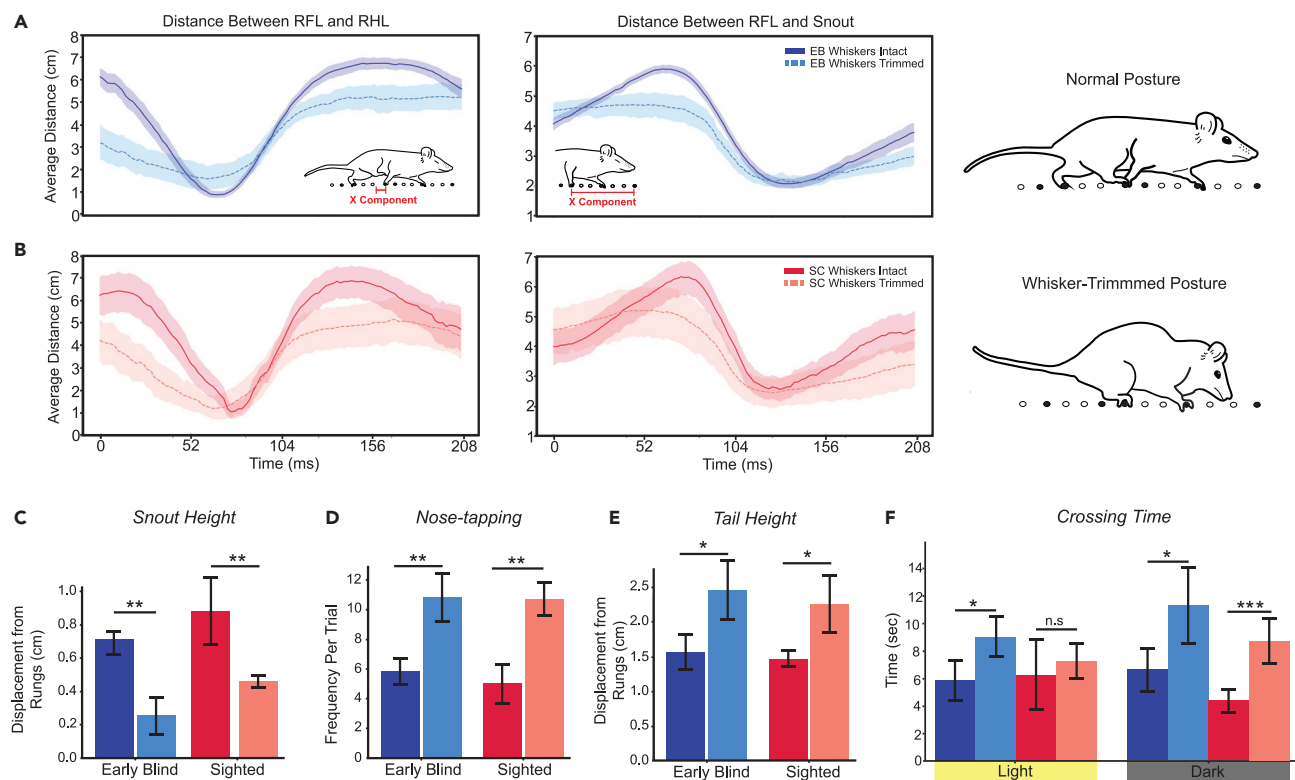


Figure 5. Animals Adapt to Whisker Trimming in Similar Ways

(A and B) (A) Average line graphs with 95% confidence intervals of stride (x component only) between the right forelimb and right hindlimb (left) and the right forelimb and snout (right) in early blind (A) and sighted (B) opossums during correct forelimb strikes. In both groups, whisker trimming reduces stride and decreases the distance between the right forelimb and snout. The flattened whisker-trimmed trajectories illustrate the conservative approach taken by whisker-trimmed opossums, as animals hunch their posture and are less willing to take long strides. Illustrations (right) derived from tracings of animals with (top) and without (bottom) whiskers, representative of typical locomotor postures while ladder crossing.

(C–E) Bar graphs depict quantified aspects of body posture during ladder crossing. (C) Whisker trimming results in significant decreases in average snout height ($p = 0.004$) and (D) more nose tapping behavior ($p = 0.002$), (E) while the tail is held higher ($p = 0.042$).

(F) Bar graph shows the interaction between whiskers and lighting condition on crossing time. Whisker trimming results in increased crossing time for early blind animals in the light and dark ($p = 0.022$, $p = 0.015$). Whisker trimming only results in increased crossing time for sighted animals in the dark ($p = 0.448$, $p < 0.001$).

Bar graphs presented as mean with bootstrapped 95% confidence intervals. Where p values are reported, statistical tests were calculated using linear models and multiple comparisons corrected by the Holm-Sidak method (see [Transparent Methods](#): reduced model). (* $p < .05$, ** $p < .01$, *** $p < .001$, n.s = $p > .05$). See also [Figure S3](#).

snout, during correct motions. Animals with whiskers, regardless of blind or sighted condition, show standard locomotor postures, with limbs oscillating between long (>5 cm) and short distances (~1 cm), reminiscent of the quadrupedal gait cycle ([Figure 5A](#) and [5B](#) solid lines) ([Videos S2](#) and [S4](#)). However, whisker-trimmed opossums show flattened trajectories, which never reach large amplitudes ([Figures 5A](#) and [5B](#) dotted lines). Instead, these condensed limb distances illustrate the hunched, conservative approach that blind and sighted animals employed when crossing the ladder without whiskers ([Videos S3](#) and [S5](#)).

Additionally, animals without whiskers held their snout closer to the rungs on average ($R^2_{adj} = 0.59$, $F(4) = 6.46$, $p = 0.004$, $\Delta_{EB} = -0.46$ cm, $\Delta_{SC} = -0.42$ cm) ([Figure 5C](#)) and exhibited significantly more nose tapping behavior ($R^2_{adj} = 0.34$, $F(4) = 6.46$, $p = 0.002$, $\Delta_{EB} = +3.89$ taps, $\Delta_{SC} = +4.56$ taps) ([Figure 5D](#)), possibly in order to gain tactile and/or olfactory information. Whisker trimming was also accompanied by an increase in average tail height during correct placements ($R^2_{adj} = 0.51$, $F(4) = 4.95$, $p = 0.042$, $\Delta_{EB} = +0.90$ cm, $\Delta_{SC} = +0.78$ cm) ([Figure 5E](#)) and a dramatic increase in crossing time ($R^2_{adj} = 0.24$, $F(4) = 7.672$, $p = 0.001$, $\Delta_{EB} = +4.86$ s, $\Delta_{SC} = +3.40$ s) ([Figures 5F](#) and [S3B–S3F](#)).

To further examine how sighted and blind animals adapted to whisker trimming, we analyzed crossing time in both lighting conditions. Proving an effective control, we found that whisker trimming increased crossing time for early blind animals irrespective of lighting condition ($p_{\text{light}} = 0.022$, $p_{\text{dark}} = 0.015$, $\Delta EB_{\text{light}} = +4.00\text{s}$, $\Delta EB_{\text{dark}} = +5.86\text{s}$) (Figure 5F). However, whisker trimming only increased crossing time for sighted controls in the dark, suggesting that whisker-trimmed sighted animals employ the use of visual cues when visual information is available ($p_{\text{light}} = 0.45$, $p_{\text{dark}} < 0.001$, $\Delta SC_{\text{light}} = +1.27\text{s}$, $\Delta SC_{\text{dark}} = +5.44\text{s}$) (Figure 5F). These metrics indicate that early blind and sighted animals adopt a conservative strategy to ladder crossing when tactile sensory input from the whiskers is removed and that sighted animals recruit vision in the absence of whiskers.

DISCUSSION

We quantified the extent to which early vision loss impacts movement strategy and performance on a sensorimotor task involving the spared senses. Our results indicate that early blind (EB) animals have superior performance on the ladder rung task, in part due to increased precision of sensory-guided forelimb placement. Whisker trimming nullified this advantage, demonstrating that EB animals relied more heavily on input from the whiskers to complete the task than sighted animals (SC). Following whisker trimming, both EB and SC animals adopted similar crossing strategies during the dark condition, but SC animals showed no increase in crossing time in the light condition (whereas EB animals did), suggesting that sighted animals utilized vision when tactile input from the whiskers was unavailable. We first discuss the increased performance of early blind animals on the ladder rung task and compare this with studies in humans that demonstrate cross-modal behavioral plasticity following the early loss of vision. We then discuss the importance of active sensing with the whiskers for locomotion and navigational tasks. Finally, we explore the underlying neural mechanisms that may subserve these sensorimotor abilities and how they have been modified following the early loss of vision.

Cross-Modal Behavioral Plasticity following Early Loss of Vision

Recent data from our laboratory indicate that EB animals are better at making fine tactile discriminations but show no differences in whisking behavior compared with SC opossums (Ramamurthy et al., *Society for Neuroscience Abstracts*, 2018; Ramamurthy and Krubitzer, 2018). In the current study, we found that EB opossums could detect rungs and place their limbs more accurately during ladder rung walking than SC opossums. Although few other animal studies have been conducted, research in humans has extensively examined the behavioral effects of the early loss of vision. Mainly, these studies have focused on two types of behavior: sensory acuity and spatial information processing. For example, it has been shown that EB individuals have increased auditory and tactile spatial acuity, reacting to auditory and tactile spatial targets quicker than SC without changes in performance (Collignon et al., 2009; Collignon and De Volder, 2009; Ptitto et al., 2005, 2008; Ricciardi et al., 2014). Using a somatosensory spatial discrimination task, Wong and colleagues found that EB individuals have higher tactile acuity on the fingers than SC but tactile discriminations made with the lips were the same in both groups (Wong et al., 2011). EB individuals also have quicker reaction times and increased accuracy on texture but not shape discrimination tasks (Gurtubay-Antolin and Rodriguez-Fornells, 2017; Schubert et al., 2017; Wan et al., 2010).

Although blind individuals use input from multiple senses to navigate, most studies of navigation in the early blind focus on the auditory system (Bedny et al., 2011; Gizewski et al., 2003; Schinazi et al., 2016). Given the prevalence of EB individuals who navigate their daily environment with a cane, it is surprising that more research has not been dedicated studying spatial navigation through tactile input. Moreover, studies that do employ tactile information to study spatial information processing in the blind often used reaction time and measured spatial acuity based on stimulus detection only (Fiehler and Rosler, 2010; Leo et al., 2012; Ricciardi et al., 2014). That is, participants in these studies only had to detect a spatial stimulus but were not required to reach toward or touch the target, neglecting potential differences in movement strategy and motor performance.

An important contribution of the present study is that we not only look at performance but also quantify the strategy adopted by our different groups when performing the ladder rung task. In a recent review, Schinazi and colleagues stress the importance of studying the relationship between strategy and performance in uncovering differences in spatial navigation abilities between congenitally blind and sighted humans (Schinazi et al., 2016). In our study, EB opossums had to detect an upcoming rung, accurately place their forelimb on the rung, and maintain a spatial representation of that rung in order to ensure accurate hindlimb

placement. We found that EB opossums had greater precision in targeting upcoming rungs with both the fore- and hind limbs while showing minor differences in limb trajectory. This superior performance was presumably due to input from the spared senses, such as the whiskers. When this spared sensory input was removed by trimming the whiskers, both EB and SC animals adopted similar crossing strategies but EB animals showed greater deficits in performance and posture.

Information on the location and distance of gaps is usually provided by the whiskers (Arkley et al., 2017); however, both blind and sighted animals adjusted to whisker trimming by using somatic and possibly olfactory input from the nose, shown by a 2-fold increase in nose-tapping behavior. Interestingly, a recent study in rats found that whisker-trimmed animals also used nose tapping to detect the location of the reach slit during a single-pellet reaching task (Parmiani et al., 2018).

Further quantification of body posture showed that animals in both groups adapted to whisker trimming by taking a more cautious approach, illustrated by a truncated stride length and shortened distance between the snout and forelimb, as well as increased crossing time in most conditions. Of note is that whisker trimming did not cause a significant increase in crossing time for sighted opossums in the light condition, whereas crossing time did increase for SC in the dark condition, indicating that SC animals may have used the visual system to perform this task when the whiskers were removed. In agreement with Shinazi and colleagues, our results show the importance of studying both performance and strategy, as one or the other may be altered depending on the available sensory information. In our case, without tactile information from the whiskers, EB opossums qualitatively resembled a blind human trying to navigate without a cane and SC opossums resembled a sighted human trying to navigate in the dark.

Whiskers Guide Forelimb Movements in Whisking Mammals

Active sensing with the whisker system has been shown to be critical for many animals to detect walls and objects and is thought to directly guide forelimb placement during locomotion (Arkley et al., 2017, 2014; Grant et al., 2013, 2009; Mitchinson et al., 2007). In the current study, we found that whisker trimming resulted in impairments in sensory-guided stepping during ladder crossing, regardless of whether opossums were blind or sighted. These results add support to the prevailing theory that whisking is fundamental for guiding limb placement. First and foremost, we found that animals without whiskers had increased forelimb error but not hindlimb error. Second, in agreement with work on precision stepping in cats and rats (Drew and Mari-gold, 2015; Whitlock, 2014), where spatial information from the forelimb informs the future trajectory of the hindlimb (by encoding the height and location of obstacles), we found that whisker trimming altered the height of both the forelimb and hindlimb. Although both groups of animals exhibited a reduction in forelimb and hindlimb peak height due to whisker trimming, this occurred to a greater extent in EB animals. Moreover, hindlimb trajectory, but not performance, was altered by whisker trimming. Third, recent research in rats has shown that whisker trimming results in increased variation in limb kinematics during locomotion on a continuous substrate (Niederschuh et al., 2015). Similarly, we found that whisker trimming resulted in more variable forelimb movements during the retraction but not extension phase of the trajectory. We believe the retraction phase to be associated with sensing an upcoming rung, as the nose oscillates vertically during correct placements. Although we did not find notable differences in the periodicity of nose oscillations (data not shown), future studies that directly examine the relationship between whisking frequency/contact and forelimb placement will shed light on this aspect of locomotor control. Regardless, increased variance in the retraction but not extension phase may indicate increased hesitation when opossums are detecting an upcoming rung's position, but once located, can make a precise placement.

Across metrics, we found that EB animals show a trend for lower variation. Although this speaks to the increased precision of whisker-guided forelimb movements in blind opossums, we cannot rule out visual input altering attention in sighted animals. Nevertheless, in the presence or absence of vision, forelimb movement types cluster into similar stereotyped movements. On the other hand, we found that the absence of whiskers forces forelimb placement trajectories to conform to similarly stereotyped movements in blind and sighted opossums. Thus, it was the loss of whiskers, and not the loss of vision, that impacted the produced stereotyped forelimb movements.

Neural Mechanisms that May Subserve Adaptive Cross-Modal Behavioral Plasticity

Following the early loss of vision, cortical areas associated with both the lost sense as well as spared sensory systems are profoundly affected. In EB mice, rats, and cats, the connections of primary visual cortex are

drastically altered, such that cortico-cortical projections from somatosensory and auditory areas densely project to what would have been visual cortex (Berman, 1991; Dye et al., 2012; Laemle et al., 2006; Negyessy et al., 2000). Studies in our laboratory demonstrate that, in enucleated opossums, V1 receives input from somatosensory areas of the cortex and thalamus and that cortical connections of S1 are altered as well (Dooley and Krubitzer, 2019; Karlen et al., 2006). Similar to studies in animal models, indirect measures of connectivity such as resting state fMRI and diffusion tensor imaging (DTI) in EB humans show that S1 is more densely connected (correlated) with V1 and that posterior parietal cortex exhibits denser projections with sensory areas as well (Klinge et al., 2010; Liu et al., 2007; Ptito et al., 2008, 2005; Shu et al., 2009; Wittenberg et al., 2004).

Functional changes accompany these anatomical changes in early blind animals and humans. Visually deprived mice and rats have altered excitatory synaptic function in V1, and neurons in a large proportion of the reorganized primary visual cortex respond to auditory stimuli (Goel et al., 2006; Piche et al., 2007; Zheng et al., 2014). In early blind *Monodelphis*, all of what would be visual cortex (V1) is co-opted by the somatosensory and auditory systems (Kahn and Krubitzer, 2002). Although the functional organization of posterior areas in parietal cortex has not been extensively explored in *Monodelphis*, research in rats and cats shows that activity in posterior parietal cortex increases before and during gait modifications (Beloozerova and Sirota, 2003; Whitlock, 2014). Importantly, in blind rats, neurotoxic lesions to posterior parietal cortex cause deficits in spatial memory during maze running (Pinto-Hamuy et al., 2004). Functional changes due to early vision loss have been extensively studied in humans. In EB individuals, V1 is activated during somato-motor tasks involving the hands (Gizewski et al., 2003) and during tasks involving memory, spatial processing, and language (Amedi et al., 2003; Bedny et al., 2011; Ricciardi et al., 2014). Visual cortex is also activated by guided hand motions, regardless of whether participants are SC or EB (Fiehler and Rosler, 2010). Furthermore, in congenitally blind humans, posterior parietal cortex retains its role as an encoder of the spatial position of a reach target and shows increased integration of tactile information (through variability of the BOLD signal) in participants completing a tactile spatial discrimination task (Leo et al., 2012; Lingnau et al., 2014).

Taken together, studies in animal models and humans demonstrate that visual cortex is not dysfunctional in the absence of vision but instead contributes to a number of behaviors including tactile and spatial processing (for review, Ricciardi et al., 2014). These studies also suggest that superior performance on tactile discriminations may be due to changes in the structure and function of somatosensory and posterior parietal cortices. Given previous data from our laboratory in EB opossums showing (1) a somatosensory-driven reorganization of V1, (2) dense projections from somatosensory cortex to V1 and thalamic motor nuclei, (3) alterations in neural response properties in the whisker representation in S1, and (4) increased connections between S1 and posterior parietal cortex, we posit that these neural changes could support the heightened abilities of EB opossums observed in this study and also explain the extreme detriments to performance when the whiskers were trimmed. Although we have focused on the cortical mechanisms that may account for differences in performance between EB and SC animals, it is possible that subcortical changes as well as alteration in the morphology of the whiskers may contribute to differences in performance, as seen in visually deprived mice and cats who have increased diameters of macro-vibrissae (Rauschecker et al., 1992). It may also be possible that EB animals are more efficient in their acquisition of sensory information through differences in whisker movement. Previous data from our laboratory indicate this is not the case, as EB and SC *Monodelphis* have similar whisker set-points and whisking frequencies (Ramamurthy and Krubitzer, 2018). Research in functionally blind rats with similar results adds support for EB animals having heightened sensory coding versus heightened sensory acquisition (Arkley et al., 2014). These data have implications for congenitally blind humans, suggesting that sensory substitution devices that rely on tactile input and behavioral therapies that involve the rapid acquisition of spatial information through tactile input will be effective in increasing the ability of EB individuals to navigate in a complex environment.

Limitations of the Study

A concern of the present study is that we do not directly show that cross-modal changes in the brain due to the early loss of vision are responsible for the observed heightened performance of EB animals. Although we do not claim causation, decades of prior research from our laboratory in the same experimental model and the rigorous quantification of behavioral strategy, posture, and performance in the present study support the hypothesis that cross-modal changes to the brain due to the early loss of vision lead to enhanced

sensorimotor performance on tasks involving the spared senses. The present study describes the phenomena that allow future work, through targeted inactivation experiments, to probe the individual contributions of cross-modal changes to S1, V1, and PPC to sensorimotor behavior.

Another limitation of this study was that we did not quantify how opossums grasped each rung or how whisker movements preceded forelimb movements. We believe both of these aspects of behavior to be critical for locomotion in whisking mammals but chose to focus on macro-scale aspects of body posture, as none of these had been described before. Previous research in blind rats, and recent data from our laboratory in EB opossums, shows that there is no difference in active whisking between blind and sighted animals (Arkley et al., 2014; Ramamurthy and Krubitzer, 2018). However, we note that possible differences in whisking behavior could exist, as this task causes constant changes in head and limb positioning. Future research on how forelimb trajectories match the frequency of whisking will elucidate how the whiskers guide individual movements.

A third limitation of this study was our use of a 660-nm lamp. Although this is outside the range of sensitivity for even the longest wavelength cone that *Monodelphis* possesses ($\lambda = 500\text{--}570\text{ nm}$), we cannot completely rule out that rods did not capture any light. In *Monodelphis*, the retinal rod pathway has sensitivity ranges between 400 and 600 nm and closely resembles that of placental mammals (Bowmaker and Dartnall, 1980; Hunt et al., 2009; Lutz et al., 2018). However, peak lamp power at 660 nm does not mean there is no detectable illumination outside of the detectable range for *Monodelphis*, and therefore it is likely that opossums did receive some form of visual input from this low energy light—yet performance data from pilot experiments (see [Transparent Methods](#)) found no difference in total error whether the lamp was on or off. Testing the illuminance of the red lamp at the ladder apparatus also revealed extremely low-energy light. Therefore, we find it unlikely that form-vision via rods contributed to sighted animals' ability to cross the apparatus in the dark. Although eye movements are well studied in primates, future studies in rodents that quantify how eye movements are related to forelimb control will elucidate if active vision guides forelimb control in whisking animals, as this is currently thought to rely on olfactory and somatosensory cues alone (Klein et al., 2012).

Resource Availability

Lead Contact

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Leah Krubitzer (lakrubitzer@ucdavis.edu).

Materials Availability

This study did not generate any new unique materials.

Data and Code Availability

The datasets and code generated during this study are available at: https://www.github.com/maceng4/Monodelphis_Ladder_Rung.

METHODS

All methods can be found in the accompanying [Transparent Methods supplemental file](#).

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.isci.2020.101527>.

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AUTHOR CONTRIBUTIONS

Conceptualization, M.E. and L.K.; Methodology, M.E.; Investigation, M.E., S.F., C.S.I., and L.K.; Writing and Editing, M.E. and L.K.; Funding Acquisition, L.K.; Supervision, L.K.

DECLARATION OF INTERESTS

No competing interests declared.

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Supplemental Information

**Available Sensory Input Determines Motor
Performance and Strategy in Early Blind
and Sighted Short-Tailed Opossums**

Mackenzie Englund, Samaan Faridjoo, Christopher S. Iyer, and Leah Krubitzer

Supplemental Figures

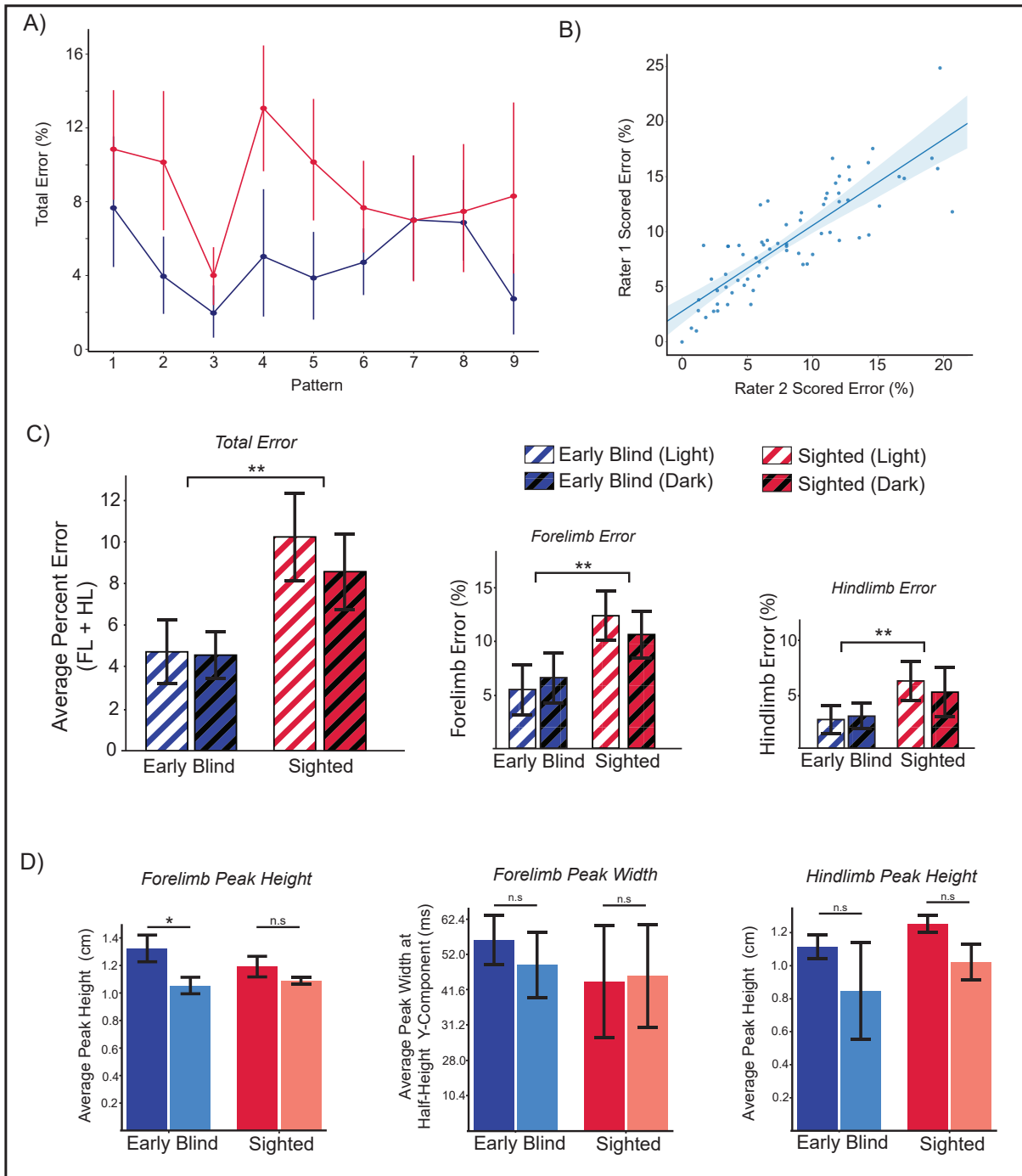


Figure S1. Ladder rung performance during different lighting conditions and quantification of limb trajectories. Related to Figures 2 and 3. (A) Average error by rung placement pattern in early blind (blue) and sighted (red) opossums. Whisker-trimmed conditions are represented by lighter shades. Early blind animals exhibit less error on average on 8 out of 9 patterns. We generated a linear model testing for the effects of pattern and order of pattern completion (dark first or light first) on total error, and found that neither significantly predicted rung walking error ($R^2_{adj} = .086$, $F(3) = 7.997$, $p = .367$, $p = .207$). (B) Scatterplot with fit line showing the degree of agreement between Rater 1 and Rater 2 on scoring variable ladder rung walking ($R^2_{adj} = .712$, $F(1) = 174.2$, $p < .001$). Interclass correlation was used to measure inter-rater reliability (Single fixed raters: $ICC3 = 0.84$, $p < .001$). (C) Average rung walking error in light (white stripes) and dark (black stripes) lighting conditions. Under the full model (see methods) no significant main effect was found for lighting condition ($R^2_{adj} = .32$, $F(8) = 6.624$, $p = .718$). (D) Bar graphs depicting quantification of limb trajectories using the reduced model and Holm-Sidak method for multiple comparisons. (Left) Average forelimb peak height is significantly reduced in early blind ($R^2_{adj} = .701$, $F(2) = 8.03$, $p = .016$) but not sighted opossums ($R^2_{adj} = .236$, $F(2) = 2.24$, $p = .119$) after whisker trimming. (Middle) Forelimb peak width at half height is not significantly altered due to whisker trimming ($R^2_{adj} = -.307$, $F(4) = 0.973$, $p = .696$). (Right) Hindlimb peak height is not significantly altered due to whisker trimming ($R^2_{adj} = .234$, $F(4) = 2.144$, $p = 0.083$). (* $p < .05$, ** $p < .01$, *** $p < .001$, n.s = $p > 0.05$). Data presented as mean with bootstrapped 95% confidence intervals.

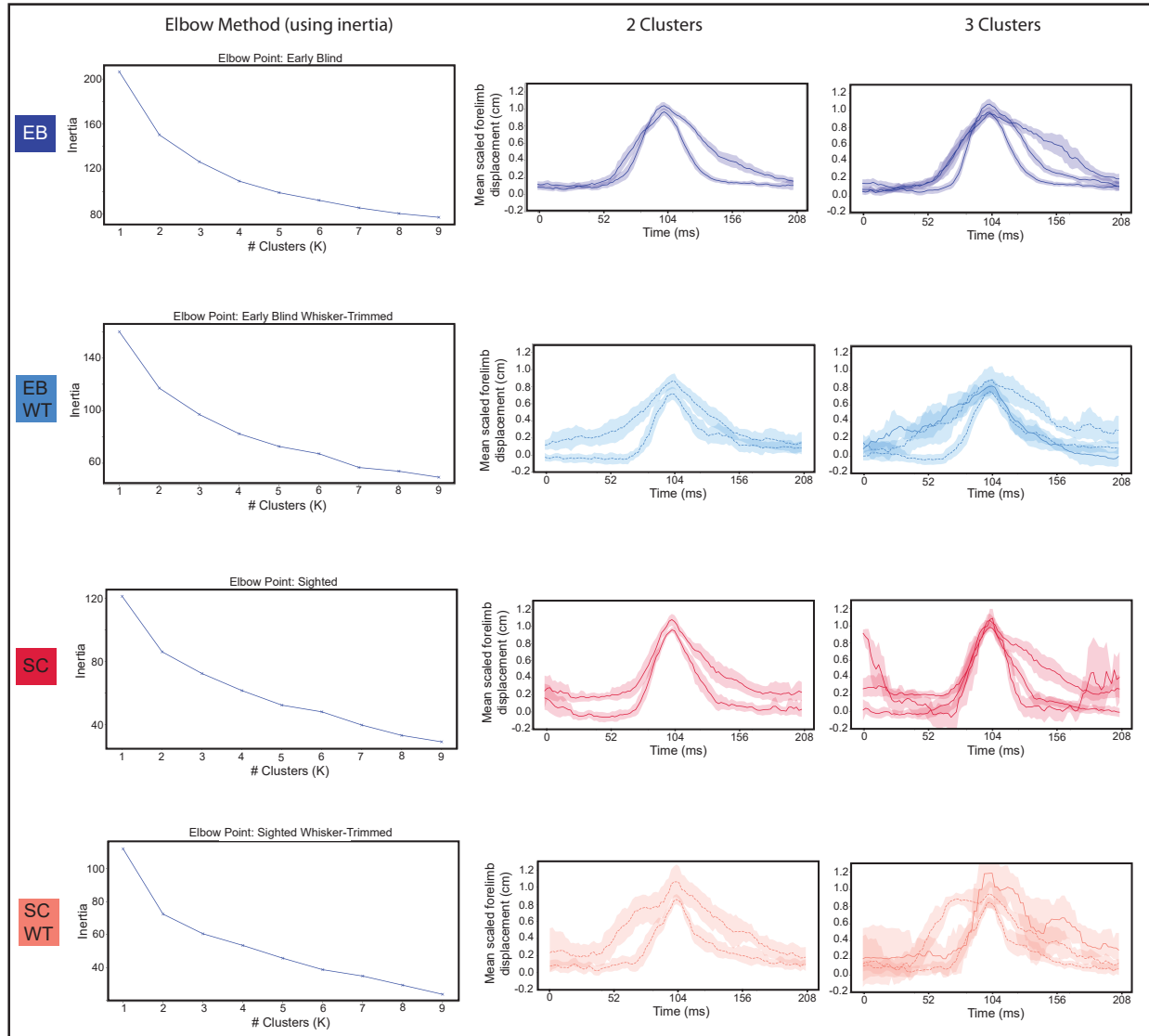


Figure S2. K-Means clustering provides stereotypical forelimb movement types during correct placements. Related to Figure 4. Column 1: The elbow point method and silhouette coefficient were used to determine the number of clusters, and thus the number of stereotyped movements for a given experimental condition. We chose the number of clusters after exponential decay ceased, and/or the silhouette coefficient was closest to 1. Columns 2 and 3: line graphs depicting average forelimb y-component per cluster with 95% confidence intervals. All movements were assigned to both 2 and 3 clusters to illustrate how movements change before and after whisker trimming, regardless of cluster number. Column 2: When 2 clusters are

used, the most notable difference is seen in the first half of one movement type (0 – 104ms) in whisker-trimmed conditions, where the forelimb is raised, but takes a linear trajectory to the peak. The other type of movement is noted to be a shallow and quick step. Column 3: When 3 clusters are used to stereotype movements, this difference is still present.

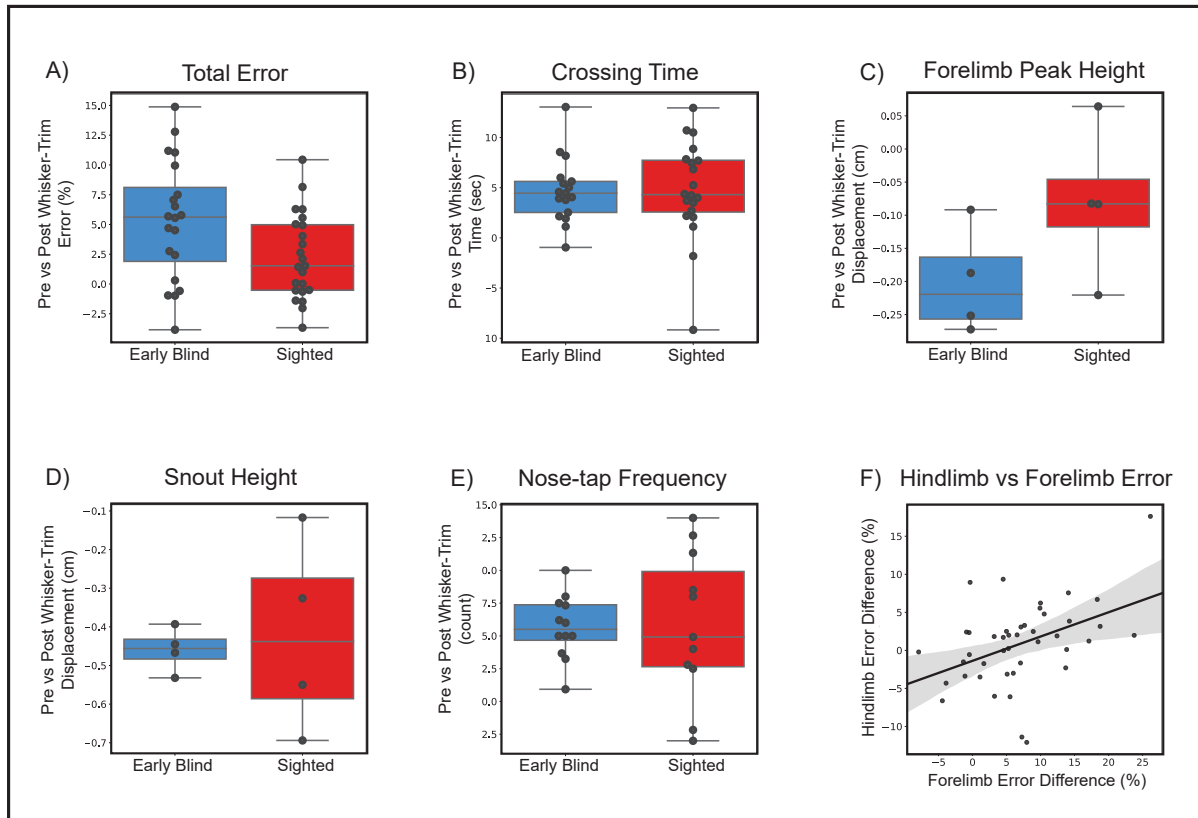


Figure S3. Within-subject differences due to whisker trimming. Related to Figure 5. (A-E)

Box and whisker plots show average change in performance, posture, and strategy due to whisker trimming. Box plots show within-group mean and 95% confidence intervals while dots represent within-subject score changes. The Y-axis denotes the difference between pre-trim and post-trim scores (Δ_{EB} / Δ_{SC}). Positive values indicate increases due to whisker trimming. Note that the directionality of the effect (either > 0 or < 0) is similar across individuals and condition, but that the magnitude differs, indicating that all individuals are affected by whisker trimming in similar ways but to varying extents. (F) Line plot with regression line and 95% CI showing that individual opossums that have greater deficits in forelimb placement due to whisker trimming also have greater deficits in hindlimb placement ($R^2_{adj} = 0.17$, $F(1) = 9.61$, $p < 0.003$; reduced model).

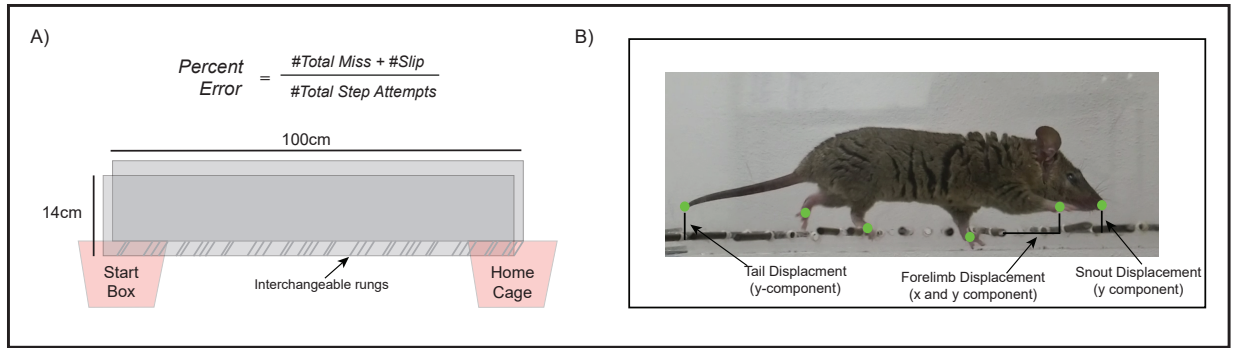


Figure S4. Variable ladder rung walking apparatus and placement of DeepLabCut tracking markers. Related to Figures 2 and 3. (A) Equation for error rate (top) and ladder rung apparatus (bottom). During a single trial, opossums move from a neutral start box (left) to their home cage (right). (B) Image of a sighted opossum on the ladder rung apparatus with illustrations of locations chosen for posture analysis.

Transparent Methods

Experimental Model and Subject Details

Animals:

Twenty-seven adult (≥ 180 days) short-tailed opossums (*Monodelphis domestica*) were used to test variable ladder rung walking. Twelve animals (5 male, 7 female) were bilaterally enucleated on postnatal day 4 (P4), while fifteen animals (8 male, 7 female) were sighted littermate controls. Four animals from each group were also used for limb tracking analysis. All animals were obtained through our breeding colony at the University of California, Davis. Animals were reared in standard laboratory conditions, weaned at 2 months, and separated from littermates at 4 months. Upon separation, females were co-housed with one female littermate, while males were single-housed. All experimental procedures were approved by UC Davis IACUC and conform to NIH guidelines.

Bilateral Enucleation Surgery:

Bilateral enucleations on *Monodelphis* pups in our laboratory have been described in detail previously (Kahn and Krubitzer, 2002; Karlen and Krubitzer, 2009). In brief, on postnatal day 4 (P4), mothers were first anesthetized with isoflurane (5%), and anesthesia was maintained with Alfaxalone (3 mg/kg, 10 mg/ml IM). Pups were anesthetized via hypothermia. At this age, pups are fused to the mother's nipple, and do not detach until around the third postnatal week. Under microscope guidance, the skin covering the retina was incised and retracted and the immature retina was removed. A flush of sterile saline was then applied to rinse the area, and the skin was replaced and sealed with surgical glue. Approximately half of each litter was bilaterally enucleated in this manner, while the other half served as littermate controls. Once enucleation procedures were complete, mothers were allowed to recover in their home cage.

Method Details

Behavioral Testing:

When enucleated and control animals reached maturity (≥ 180 days), we tested their performance on the variable ladder rung walking task, commonly used in rodents (Schönfeld et al., 2017). Animals were first tested in both light and dark conditions (in varying order) with their whiskers intact. We used a 660nm lamp (outside the peak-sensitivity range for cones in *Monodelphis*) to ensure accurate video collection and scoring in the dark condition (Hunt et al., 2009; Seelke et al., 2014). Pilot studies ($n = 4$ EB, $n = 4$ SC) from our lab in complete darkness, where a camera with an infrared sensor and no red lamp were used for the dark condition (along with manual frame-by-frame scoring) show there was no effect of the presence of the red lamp on total error in EB or SC opossums ($R^2_{\text{adj}} = 0.32$, $F(7) = 8.36$, $p = 0.83$, $\Delta\text{EB}_{\text{lamp on vs off}} = -0.46\%$, $\Delta\text{SC}_{\text{lamp on vs off}} = +0.29\%$). To further confirm that the red lamp did not affect testing, we measured illuminance using a Digital Illuminance Light Meter LX1330B, as lux provided by the red lamp during the dark condition at the ladder apparatus, 1.8 meters away from the light source. Our measurements indicated that 0.1 lux was detectable at this location. This is similar to natural levels of darkness (new moon ~ 0.001 lux, full moon ~ 1 lux), but not pitch black (0 lux) (Seelke et al., 2014). From lux, sensor size, and bulb type, we calculated power at this location to be $6.64e-6$ Watts. As the presence of the red lamp did not significantly affect performance, all remaining testing was completed with the lamp on.

At least one week after initial testing, all mystacial, submandibular, and genal whiskers were trimmed and animals were tested again in both lighting conditions on two consecutive days. To trim the whiskers, animals were briefly anesthetized with isoflurane (2-5%) and macrovibrissae were trimmed down to ~ 1 mm using an electric clipper. Animals were allowed to recover for 12 hours in their home cage before undergoing testing. All whisker-trim testing was conducted

within 72 hours of trimming to prevent animals from recovering whisker sensation due to significant regrowth.

Variable Ladder Rung Walking:

The ladder rung apparatus consisted of an elevated trough with a floor of ladder rung-like pegs on which the animals could walk (Figure S4A). The trough was constructed from two Plexiglas walls 1 meter in length, connected by a floor of metal pegs (3 mm diameter, 10 cm wide) similar to that used by Metz and Whishaw (Metz and Whishaw, 2009). Holes for the removable rungs forming the floor of the apparatus were spaced at 1cm intervals. The apparatus was supported by a neutral start box on one end and the animal's home cage on the other end, and was raised approximately 1 meter from the floor to discourage jumping. Animals crossed the apparatus from the neutral start cage to their home cage. Motivation to reach the home cage was high, so no additional reward was given.

For the baseline (standard pattern) condition, all rungs were equally spaced 2 cm apart. For variable patterns, rungs were spaced 1 - 5 cm apart, with no more than 3 rungs of 1 cm spacing in a row before a 2 cm gap. Variable patterns were generated using a custom Python program which determined random 10-rung patterns, which were then repeated over the 1-meter long apparatus. To capture naturalistic behavior, without effects of training, each animal participated in only five days of testing. Day 1 consisted of five runs of the standard pattern to allow for habituation, with all rungs at 2 cm spacing. Days 2 - 5 consisted of three standard patterns per animal, followed by two or three variable patterns. Two consecutive days of testing were done with lights on (either days 2&3 or days 4&5) on variable patterns (light condition), and two consecutive days of testing were done in 660 nm red light (dark condition). Trials were video-recorded and scored (see below). Trials in which animals did not cross at least half (0.5 m) of the apparatus before stopping or reversing direction were not scored (< 5% of total trials).

Scoring:

We adapted the scoring method originally used by Metz and Whishaw (Metz and Whishaw, 2009). The four-point scale included: (0) Total Miss: 0 points were awarded when a limb entirely missed a rung, and body posture was disturbed. (1) Slip: 1 point was awarded when the limb initially contacted a rung before slipping off. (2) Correction/Replacement: 2 points were awarded when the limb aimed for one rung, but was placed on a different rung before contacting the first, or when the limb was placed on one rung, but moved to another rung before weight-bearing. (3) Correct Placement: 3 points were awarded when a limb was advanced and placed on a rung and could bear weight without causing a visible disturbance in body posture. Scores of (0) and (1) were combined as errors and divided by the total number of attempts to produce an error rate (Figure S4A). Crossing time was also scored, excluding time when the animal would stop to groom.

Because our initial observations showed animals changing head pitch to tap their nose on the next rung before stepping, we quantified the frequency of nose taps in each trial as state events, when the rostrum made physical contact with a rung. Scores for foot fault, crossing time, and nose taps were averaged by animal across all variable patterns.

Manual video analysis:

Videos were recorded with various cameras to allow for wide-field, standard, and high-speed video capture from multiple angles. A Canon VIXIA HF R500 camcorder (60 fps) and GoPro Hero 6 (240 fps) were used to record light trials, while a SEREE FHD 1080P camcorder (30 fps), was used to record dark trials. Videos were scored in VLC Player using frame-by-motion, and at 25 and 50% of standard playback speed (7.5 – 30 fps) by independent raters and/or using the DeepLabCut analysis system (see below). Two observers blind to the animal's condition scored all trials independently. Scores were averaged between observers to provide a single measure (for inter-rater reliability measures see Statistics; Supplementary Figure 1).

Automated video analysis:

Video of ladder rung crossing strategy was also analyzed by DeepLabCut, a marker-less pose-detecting machine learning Python package (Mathis et al., 2018). This toolbox utilizes Google Tensorflow and ResNet to track any user-defined body part, animal, or object in successive video frames. DeepLabCut is blind to experimental condition, and is capable of tracking movements that allow us to quantify and analyze differences in strategy. Videos were captured perpendicular to the ladder apparatus, using a fixed position in order to capture the speed of the animal and not interfere with an animal's attention during crossing. All videos were cropped and downsized to size 300x900 pixels to meet DeepLabCut size requirements. We trained the neural network for 400,000 iterations on 300 labeled frames. This regimen was adequate to produce the desired fit of the model to the training data (loss $<.005$), such that predictions of movements by DeepLabCut resulted in accurate tracking of body parts (Supplemental Video 1). Custom Python programs were produced to analyze per-frame positional information of all four limbs, the tail, and snout, during light and dark trials (code available at: https://www.github.com/maceng4/Monodelphis_Ladder_Rung).

To study changes in locomotor patterns as opossums crossed the ladder apparatus, we restricted our analysis to correct placements of the right forelimb, during which: 1) no other limbs were currently making an error and 2) all DLC trackers accurately labeled the assigned body part for the duration of the gait cycle. Both a human observer and computer vision (continuous plots of each tracker) were used to provide confirmation. Correct strikes were defined as motions of the right forelimb which began on a single rung and involved reaching for and correctly grasping a new rung. These motions corresponded to performance scores of 3: correct placement. We selected 100 video frames centered on the peak of each forelimb movement using the above criteria (50 frames prior to the peak and 50 frames after, captured at

120 x 4 fps). Consequently, each analyzed strike yielded 208 milliseconds of data of the whole body during a correct placement of the right forelimb.

Data across all strikes was aligned to the highest peak during forelimb motion, aggregated along the time axis (208 milliseconds) and separated into X and Y displacement components to provide average trajectories per group. The Y-component represents height (displacement from the ladder apparatus in centimeters) and the X-component represents step length (Figure S4B). To determine differences between groups, we characterized trajectories by quantifying peak width at half-height, average peak height, average displacement, and binned variance. We accounted for differences in animal size by scaling all data for an individual animal by its average forelimb height during a correct movement, before combining data across animals. Statistical models tested for differences between experimental conditions using per-animal averages.

For analyses of stereotyped movements (K-means clustering), we generated scripts which implemented supervised machine learning packages from sci-kit learn (sklearn). We used the elbow method in concert with the silhouette coefficient (described below) to select the number of clusters (movement types) that a given forelimb movement could be assigned to (Supplemental Figure 2) (Syakur et al., 2018; Zhou and Gao, 2014). For the elbow method, the x-value at which exponential decay ceases (i.e at the elbow joint of the line graph) estimated the optimal number of clusters to use for a given dataset. To confirm this estimation, we then used the silhouette coefficient, calculated using the mean intra-cluster distance and the mean nearest-cluster distance. In short, the silhouette coefficient is a measure of separation between groups, where a high number indicates an instance is well-matched to its own cluster. The two highest silhouette coefficients were used to select the number of clusters per experimental condition. In our case, this resulted in either 2 or 3 clusters for each condition (see Results; Supplementary Figure 2).

Quantification and Statistical analysis

Statistical tests were performed using custom Python scripts (https://www.github.com/maceng4/Monodelphis_Ladder_Rung). We generated fixed effects linear models and used analyses of variance to assess differences between groups. For all analyses, we tested multiple models using backward selection to ensure statistical accuracy. To assess differences in performance between early blind and sighted animals, we used a fixed effects linear model, testing for main effects of sightedness, lighting, biological sex, and the presence of whiskers. This model included interaction terms for the presence of whiskers (trimmed or intact) and lighting condition (light or dark), while sex was included as a covariate (see below: full model). After finding no significant main effect for lighting condition on performance (Supplemental Figure 1C), we collapsed across light and dark conditions for further analysis (see reduced model).

*Full Model: Error ~ Sightedness * Whiskers * Lighting + Biological Sex*

*Reduced Model: Outcome Measure ~ Sightedness * Whiskers + Biological Sex*

We used repeated measures ANOVAs to test for differences within and across testing days, and chose an alpha level < .05 for significance, and the Holm-Sidak method to correct for multiple comparisons. For analysis of DeepLabCut trajectory data, we used $n = 8$ animals, instead of $n = \#$ of total trajectories, in order to avoid skewing the data toward animals who had a greater number of analyzed trajectories (Supplemental Figure 1D). All bar graphs and trajectory data are presented as means with bootstrapped 95% confidence intervals. Given the variability we observe when working with wild-type strains of laboratory animals on untrained naturalistic behavioral tasks (Englund et al., *Society for Neuroscience Abstracts* 2018), we tested for normalcy in performance data using the Shapiro-Wilk test. Indeed, scores for total

error were found to be normally distributed ($n = 27$, $w = 0.979$, $p = 0.13$), as was trajectory data of forelimb peak height gathered from the subset of animals used for DLC tracking ($n = 8$, $w = 0.959$, $p = 0.648$).

To test for inter-rater reliability we first generated a linear model, finding significant agreement between raters ($R^2_{\text{adj}} = 0.712$, $F(1) = 174.2$, $p < 0.001$; Supplemental Figure 1B). Additionally, we calculated the intraclass correlation, also finding good agreement (Single fixed raters: $\text{ICC3} = 0.84$, $p < 0.001$). This test is commonly used to assess consistency when quantitative measurements (in our case error percentage) are made by different observers (Hallgren, 2012).

Supplemental References

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