

1 **Title: Spinal sensorimotor circuits play a prominent role in hindlimb locomotor recovery after**  
2 **staggered thoracic lateral hemisections but cannot restore posture and interlimb coordination**  
3 **during quadrupedal locomotion in adult cats**

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5 **Abbreviated title:** Locomotor recovery after staggered hemisections

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34

### 35 **Significance Statement**

36 Coordinating the limbs during locomotion depends on pathways in the spinal cord. We used a spinal  
37 cord injury model that disrupts communication between the brain and spinal cord by sectioning half of  
38 the spinal cord on one side and then about two months later, half the spinal cord on the other side at  
39 different levels of the thoracic cord in cats. We show that despite a strong contribution from neural  
40 circuits located below the second spinal cord injury in the recovery of hindlimb locomotion, the  
41 coordination between the forelimbs and hindlimbs weakens and postural control is impaired. We can  
42 use our model to test approaches to restore the control of interlimb coordination and posture during  
43 locomotion after spinal cord injury.

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48 **ABSTRACT**

49 Spinal sensorimotor circuits interact with supraspinal and peripheral inputs to generate quadrupedal  
50 locomotion. Ascending and descending spinal pathways ensure coordination between the fore- and  
51 hindlimbs. Spinal cord injury disrupts these pathways. To investigate the control of interlimb coordination  
52 and hindlimb locomotor recovery, we performed two lateral thoracic hemisections placed on opposite  
53 sides of the cord (right T5-T6 and left T10-T11) at an interval of approximately two months in eight adult  
54 cats. In three cats, we then made a complete spinal transection caudal to the second hemisection at  
55 T12-T13. We collected electromyography and kinematic data during quadrupedal and hindlimb-only  
56 locomotion before and after spinal lesions. We show that 1) cats spontaneously recover quadrupedal  
57 locomotion following staggered hemisections but require balance assistance after the second one, 2)  
58 coordination between the fore- and hindlimbs displays 2:1 patterns and becomes weaker and more  
59 variable after both hemisections, 3) left-right asymmetries in hindlimb stance and swing durations  
60 appear after the first hemisection and reverse after the second, and 4) support periods reorganize after  
61 staggered hemisections to favor support involving both forelimbs and diagonal limbs. Cats expressed  
62 hindlimb locomotion the day following spinal transection, indicating that lumbar sensorimotor circuits  
63 play a prominent role in hindlimb locomotor recovery after staggered hemisections. These results reflect  
64 a series of changes in spinal sensorimotor circuits that allow cats to maintain and recover some level of  
65 quadrupedal locomotor functionality with diminished motor commands from the brain and cervical cord,  
66 although the control of posture and interlimb coordination remains impaired.

67

68 **Key words:** Locomotion, Interlimb coordination, Staggered hemisections, Spinal transection, Central  
69 pattern generator, Cats

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71

## 72 **Introduction**

73 Terrestrial locomotion in mammals involves complex dynamic interactions between spinal circuits,  
74 supraspinal signals and peripheral sensory inputs [reviewed in (Rossignol et al., 2006; Frigon, 2017;  
75 Frigon et al., 2021)]. Musculoskeletal properties also play an important role in stabilizing quadrupedal  
76 locomotion and can offset some of loss in neural communication between the brain/cervical cord and  
77 the lumbar cord after spinal cord injury (SCI) (Audet et al., 2022). After complete spinal thoracic  
78 transection, hindlimb locomotion recovers in various mammals, including mice, rats, cats and dogs  
79 (Shurrager and Dykman, 1951; Barbeau and Rossignol, 1987; Bélanger et al., 1996; De Leon et al.,  
80 1998, 1999; Cha et al., 2007; Harnie et al., 2019). This recovery involves the locomotor central pattern  
81 generator (CPG) that interacts with sensory feedback from the hindlimbs (Brown, 1911; Grillner and  
82 Shik, 1973; Grillner and Zangger, 1979; Forssberg et al., 1980; Barbeau and Rossignol, 1987; McCrea  
83 and Rybak, 2008; Rossignol and Frigon, 2011; Kiehn, 2016; Grillner and El Manira, 2020; Frigon et al.,  
84 2021). Decerebrate cats with a high cervical (C1-C2) transection also express quadrupedal locomotion  
85 with pharmacology (Miller and van der Meché, 1976; Miller et al., 1977). However, it is unclear if fore-  
86 and hindlimb movements remain coordinated without supraspinal inputs (Frigon, 2017).

87 Lumbar sensorimotor circuits also play a prominent role in hindlimb locomotor recovery following  
88 incomplete SCI (Barrière et al., 2008, 2010). Barriere et al. (2008) performed a dual-lesion paradigm,  
89 consisting of a lateral hemisection at T10-T11 followed by complete spinal transection at T12-T13.  
90 Instead of taking the minimum 2-3 weeks of treadmill locomotor training usually required, hindlimb  
91 locomotion was expressed the day after spinal transection. Thus, after incomplete SCI, plasticity within  
92 lumbosacral circuits allowed them to function without motor commands originating from above the spinal  
93 transection. The lumbar locomotor CPG likely contributes to hindlimb locomotor recovery after other  
94 types of incomplete SCIs.

95 Another dual spinal lesion paradigm involves performing two lateral hemisections on opposite sides  
96 of the cord at different levels (i.e. staggered hemisections) to determine if neural communication remains  
97 possible between cervical and lumbosacral levels by activating short propriospinal pathways  
98 (Ingebritsen, 1933; Kato et al., 1984, 1985; Stelzner and Cullen, 1991; Courtine et al., 2008; van den

99 Brand et al., 2012; Cowley et al., 2015). (Kato et al., 1984) performed two types of staggered  
100 hemisections in adult cats, low thoracic followed by mid-thoracic and high cervical followed by mid-  
101 thoracic. In the two types of staggered hemisection paradigms, new fore-hind coordination patterns  
102 emerged, with the forelimbs taking more steps than the hindlimbs, or a 2:1 fore-hind coordination, with  
103 no consistent phasing between the fore- and hindlimbs during overground locomotion. These results  
104 indicate that the spinal locomotor CPGs controlling the forelimbs, located at low cervical/upper thoracic  
105 segments (Ballion et al., 2001; Yamaguchi, 2004), operated at a different rhythm and independently  
106 from those controlling the hindlimbs, located at upper to mid-lumbar spinal segments (Cazalets et al.,  
107 1995; Kiehn and Kjaerulff, 1998; Marcoux and Rossignol, 2000; Kiehn and Butt, 2003; Langlet et al.,  
108 2005). However, (Kato et al., 1984) did not separate cycles with 1:1 and 2:1 fore-hind coordination.  
109 Studies in intact and single-hemisected cats have shown that step-by-step phasing between the fore-  
110 and hindlimbs can remain consistent despite 2:1 coordination during treadmill locomotion (Thibaudier  
111 et al. 2013, 2017; Thibaudier and Frigon 2014).

112 The purpose of the present study was to determine how staggered hemisections affected the control  
113 of interlimb coordination and the recovery of hindlimb locomotion. We hypothesize that fore-hind  
114 coordination is lost following the second hemisection due to the disruption of direct communication  
115 between cervical and lumbar levels. We also hypothesize that spinal sensorimotor circuits play a  
116 prominent role in the recovery of hindlimb locomotion following staggered hemisections.

117

## 118 **Materials and Methods**

### 119 *Ethical approval*

120 The Animal Care Committee of the Université de Sherbrooke approved all procedures in accordance  
121 with policies and directives of the Canadian Council on Animal Care (Protocol 442-18). Current data  
122 were obtained from eight adult cats (> 1 year of age at the time of experimentation), 4 females and 4  
123 males, weighing between 4.1 kg and 6.5 kg ( $5.3 \pm 1.0$ ). Before and after the experiments, cats were  
124 housed and fed in a dedicated room within the animal care facility of the Faculty of Medicine and Health  
125 Sciences at the Université de Sherbrooke. Our study followed ARRIVE guidelines for animals studies

126 (Percie du Sert et al., 2020). As part of our effort to reduce the number of animals used in research, all  
127 cats participated in other studies to answer different scientific questions, some of which have been  
128 published (Lecomte et al., 2022, 2023; Merlet et al., 2022).

129

### 130 General surgical procedures

131 Surgical procedures were performed under aseptic conditions with sterilized equipment in an  
132 operating room, as described previously (Hurteau et al., 2017; Harnie et al., 2019, 2021; Audet et al.,  
133 2022). Before surgery, cats were sedated with an intramuscular injection of butorphanol (0.4 mg/kg),  
134 acepromazine (0.1 mg/kg), and glycopyrrolate (0.01 mg/kg). Ketamine/diazepam (0.05 ml/kg) was then  
135 injected intramuscularly for induction. Cats were anesthetized with isoflurane (1.5–3%) delivered in O<sub>2</sub>,  
136 first with a mask and then with an endotracheal tube. During surgery, we adjusted isoflurane  
137 concentration by monitoring cardiac and respiratory rates, by applying pressure to the paw (to detect  
138 limb withdrawal), by assessing the size and reactivity of pupils and by evaluating jaw tone. We shaved  
139 the animal's fur (back, stomach, fore- and hindlimbs) using electric clippers and cleaned the skin with  
140 chlorhexidine soap. Cats received a continuous infusion of lactated Ringers solution (3 ml/kg/h) during  
141 surgery through a catheter placed in a cephalic vein. A rectal thermometer monitored body temperature,  
142 which was maintained within physiological range ( $37 \pm 0.5^\circ\text{C}$ ) using a water-filled heating pad placed  
143 under the animal and an infrared lamp ~50 cm over it. At the end of surgery, we injected an antibiotic  
144 (Cefovecin, 0.1 ml/kg) subcutaneously and taped a transdermal fentanyl patch (25 mcg/h) to the back  
145 of the animal 2–3 cm rostral to the base of the tail to provide prolonged analgesia (4–5-day period before  
146 removal). We also injected buprenorphine (0.01 mg/kg), a fast-acting analgesic, subcutaneously at the  
147 end of the surgery and a second dose ~7 h later. Following surgery, we placed the cat in an incubator  
148 until they regained consciousness.

149

### 150 Electrode implantation

151 We implanted all cats with electrodes to chronically record the electrical activity (EMG,  
152 electromyography) of several fore- and hindlimb muscles. We directed pairs of Teflon-insulated

153 multistrain fine wires (AS633; Cooner Wire, Chatsworth, CA, USA) subcutaneously from two head-  
154 mounted 34-pin connectors (Omnetics, Minneapolis, MN, USA). Electrodes were sewn into the belly of  
155 selected fore- and hindlimb muscles for bipolar recordings, with 1–2 mm of insulation stripped from each  
156 wire. We verified electrode placement during surgery by electrically stimulating each muscle through the  
157 matching head connector channel. The head connector was secured to the skull using dental acrylic  
158 and four to six metallic screws.

159

### 160 *Staggered hemisections and spinal transection*

161 After collecting data in the intact state, a lateral hemisection was made between the fifth and sixth  
162 thoracic vertebrae on the right side of the spinal cord. General surgical procedures were the same as  
163 described above. The skin was incised between the fifth and sixth thoracic vertebrae and after carefully  
164 setting aside muscle and connective tissue, a small laminectomy of the dorsal bone was made. After  
165 exposing the spinal cord, we applied xylocaine (lidocaine hydrochloride, 2%) topically and made two to  
166 three injections on the right side of the cord. The right side of the spinal cord was then hemisected with  
167 surgical scissors between the fifth and sixth thoracic vertebrae. A hemostatic material (Spongostan) was  
168 inserted at the lesion site to stop residual bleeding, and muscles and skin were sewn back to close the  
169 opening in anatomic layers. In the days following hemisection, cats were carefully monitored for  
170 voluntary bodily functions by experienced personnel and bladder and large intestine were manually  
171 expressed as needed. The hindlimbs were cleaned as needed to prevent infection. After collecting data  
172 following the first hemisection, we performed a second lateral hemisection between the 10<sup>th</sup> and 11<sup>th</sup>  
173 thoracic vertebrae on the left side of the spinal cord nine to twelve weeks later. Surgical procedures and  
174 post-operative care were the same as following the first hemisection. After the second hemisection, we  
175 collected data for eight to twelve weeks. In three cats (TO, JA, HO), we performed a complete spinal  
176 transection at T12-T13 nine to ten weeks after the second hemisection. We did not perform spinal  
177 transections in the other cats because we had to prematurely euthanize them at the start of the covid-  
178 19 pandemic. Surgical procedures and post-operative care were the same as following the  
179 hemisections.

180 Experimental protocol

181 We collected kinematic and EMG data before (intact state) and at four different time points before  
182 and after staggered hemisections during tied-belt (equal left-right speeds) quadrupedal locomotion at  
183 0.4 m/s. The treadmill consisted of two independently controlled running surfaces 120 cm long and 30  
184 cm wide (Bertec, Columbus, OH). A Plexiglas separator (120 cm long, 3 cm high, and 0.5 cm wide) was  
185 placed between the left and right belts to prevent the limbs from impeding each other. We present data  
186 collected at weeks 1-2 and 7-8 after the first and second hemisections. Cats were not trained to recover  
187 quadrupedal locomotion but data collection included several treadmill tasks, such as tied-belt locomotion  
188 from 0.4 to 1.0 m/s and split-belt locomotion (left slow/right fast and right slow/left fast), with both the  
189 right and left sides stepping on the slow and fast belts (Lecomte et al., 2022). Cats also performed  
190 overground locomotion in a straight line and in turns on a custom-built walkway, as well as obstacle  
191 negotiations (Lecomte et al., 2023). Some projects also included having cats walk on different surfaces  
192 (e.g., foam) to evaluate the influence of somatosensory feedback. We also evoked cutaneous reflexes  
193 in some cats by stimulating the superficial radial, superficial peroneal and distal tibial nerves during tied  
194 belt and split-belt locomotion at 0.4 m/s and 0.8 m/s. In the intact state and after the first hemisection,  
195 nerves were also stimulated with longer trains to induce stumbling corrective reactions in the fore- and  
196 hindlimbs during treadmill locomotion at 0.4 m/s and 0.8 m/s (Merlet et al., 2022). Other manuscripts  
197 are in preparation. In three cats, we collected data during hindlimb-only locomotion one day, two days,  
198 one week, two weeks and three weeks after spinal transection with the forelimbs placed on a stationary  
199 platform. At two or three weeks after spinalization, we also collected data during quadrupedal treadmill  
200 locomotion at 0.4 m/s in these spinal cats.

201 In all locomotor trials and at all time points, the goal was to collect ~15 consecutive cycles using  
202 positive reinforcement (food, affection). To avoid fatigue, ~30 s of rest were given between trials. When  
203 required, an experimenter held the tail of the animal to provide mediolateral balance but not to provide  
204 weight support. In the double-hemisected and spinal states, some cats, required manual stimulation of  
205 the skin of the perineal region to facilitate hindlimb locomotion. For perineal stimulation, the same  
206 experimenter manually rubbed/pinched the perineal region with the index finger and thumb. As



207 described, the strength of perineal stimulation is difficult to quantify but we adjusted the pressure applied  
208 to the perineal region on a case-by-case basis (light/strong, tonic/rhythmic) to achieve the best hindlimb  
209 locomotor pattern possible (Caron et al., 2020; Audet et al., 2022). Perineal stimulation increases spinal  
210 excitability and facilitates hindlimb locomotion in spinal mammals through an undefined  
211 mechanism (Merlet et al., 2021). However, if the perineal stimulation was too strong, we observed  
212 exaggerated flexion of the hindlimbs (hip, knee and ankle) and/or improper left-right alternation, which  
213 impaired treadmill locomotion. In other words, too much excitability to spinal locomotor networks was  
214 detrimental.

215

### 216 Data collection and analysis

217 We collected kinematic and EMG data as described previously (Harnie et al., 2018, 2019, 2021;  
218 Lecomte et al., 2021; Audet et al., 2022). Reflective markers were placed on the skin over bony  
219 landmarks: the scapula, minor tubercle of the humerus, elbow, wrist, metacarpophalangeal joint and at  
220 the tips of the toes for the forelimbs and over the iliac crest, greater trochanter, lateral malleolus,  
221 metatarsophalangeal joint and at the tip of the toes for the hindlimbs. Videos of the left and right sides  
222 were obtained with two cameras (Basler AcA640-100 g) at 60 frames/s with a spatial resolution of 640  
223 x 480 pixels. A custom-made program (Labview) acquired the images and synchronized acquisition with  
224 EMG data. EMG signals were preamplified (10 $\times$ , custom-made system), bandpass filtered (30–1000  
225 Hz), and amplified (100–5000 $\times$ ) using a 16-channel amplifier (model 3500; A-M Systems). As we  
226 implanted more than 16 muscles per cat, we obtained data in each locomotor condition twice, one for  
227 each connector, as our data acquisition system is limited to 16 channels. EMG data were digitized (2000  
228 Hz) with a National Instruments card (NI 6032E, Austin, TX, USA), acquired with custom-made  
229 acquisition software and stored on computer. In the present study, EMG data are used only for  
230 illustrative purposes to show the gait patterns before and after spinal lesions. Measures of EMG and  
231 more detailed descriptions will be presented in upcoming papers.

232 *Temporal variables.* By visual detection, the same experimenter determined, for all four limbs, paw  
233 contact as the first frame where the paw made visible contact with the treadmill surface, and liftoff as

234 the most caudal displacement of the toes. We measured cycle duration from successive paw contacts,  
235 while stance duration corresponded to the interval of time from foot contact to the most caudal  
236 displacement of the toe relative to the hip/shoulder (Halbertsma, 1983). We calculated swing duration  
237 as cycle duration minus stance duration. Based on contacts and liftoffs for each limb, we measured  
238 individual periods of support (double, triple and quad) and expressed them as a percentage of cycle  
239 duration, as described previously (Frigon et al., 2014; Lecomte et al., 2022; Merlet et al., 2022). During  
240 a normalized cycle, here defined from successive right hindlimb contacts, we identified nine periods of  
241 limb support (Gray and Basmajian, 1968; Wetzell and Stuart, 1976; Frigon et al., 2014; Lecomte et al.,  
242 2022). We evaluated temporal interlimb coordination by measuring phase intervals between six pairs of  
243 limbs (Thibaudier et al., 2017): 1) left and right forelimbs (forelimb coupling), 2) left and right hindlimbs  
244 (hindlimb coupling), 3) left forelimb and left hindlimb (left homolateral coupling), 4) right forelimb and  
245 right hindlimb (right homolateral coupling), 5) left forelimb and right hindlimb (right diagonal coupling),  
246 and 6) right forelimb and left hindlimb (left diagonal hindlimb). Phase intervals were calculated as the  
247 absolute amount of time between contacts of two limbs divided by the cycle duration of the reference  
248 limb (English, 1979; English and Lennard, 1982; Orsal et al., 1990; Frigon et al., 2014; Thibaudier and  
249 Frigon, 2014; Thibaudier et al., 2017; Audet et al., 2022). The reference limb was always the hindlimb,  
250 with the exception of forelimb coupling where it was the right forelimb. For hindlimb coupling, the  
251 reference limb was the right hindlimb. Values were then multiplied by 360 and expressed in degrees to  
252 illustrate their continuous nature and possible distributions (English and Lennard, 1982; Thibaudier et  
253 al., 2017). To determine if single-hemisected and double-hemisected cats displayed greater variations  
254 in limb couplings, we calculated the coefficient of variation, a statistical measure of the relative  
255 dispersion of data points around the mean, by dividing the standard deviation by the mean, as we  
256 described previously (Audet et al., 2022). These values were then multiplied by 100 and expressed as  
257 a percentage.

258 *Spatial variables.* We analyzed spatial variables using DeepLabCut™, an open-source machine  
259 learning program with deep neural network (Mathis et al., 2018), as we recently described in the cat  
260 (Lecomte et al., 2021). Stride length was measured for the right fore- and right hindlimbs as the distance

261 between contact and liftoff added to the distance traveled by the treadmill during the swing phase,  
262 obtained by multiplying swing duration by treadmill speed (Courtine et al., 2005; Goetz et al., 2012;  
263 Thibaudier and Frigon, 2014; Dambreville et al., 2015; Lecomte et al., 2021). We measured the relative  
264 distance of the paw at contact and liftoff as the horizontal distance between the toe and shoulder or hip  
265 markers at stance onset and offset, respectively, for the right fore- and right hindlimbs. As an indicator  
266 of limb interference, we measured the horizontal distance between the toe markers of the fore- and  
267 hindlimbs on the same side at stance onset and offset of each of the four limbs of the animals.

268

### 269 Histology and euthanasia

270 At the end of the experiments, cats were anesthetized with isoflurane before receiving a lethal dose  
271 (100 mg/kg) of pentobarbital through the left or right cephalic vein. The extent of the spinal lesion was  
272 confirmed by histology, as described previously (Lecomte et al., 2022, 2023). Following euthanasia, a  
273 2 cm length of the spinal cord centered on the lesion sites was dissected and placed in 25 mL of 4%  
274 paraformaldehyde solution (PFA in 0.1 m PBS, 4°C). After five days, the spinal cord was cryoprotected  
275 in PBS with 30% sucrose for 72 h at 4°C. We then cut the spinal cord in 50 µm coronal sections on  
276 gelatinized slides using a cryostat (Leica CM1860, Leica Biosystems Inc, Concord, ON, Canada).  
277 Sections were mounted on slides and stained with 1% Cresyl violet. For staining, slides were then  
278 dehydrated in successive baths of ethanol 50%, 70% and 100%, 5 minutes each. After a final 5 minutes  
279 in a xylene bath, slides were left to dry before being scanned by Nanozoomer (Hamamastu Corporation,  
280 Bridgewater Township, NJ, USA). We then performed qualitative and quantitative evaluations of the  
281 lesion sites in the transverse plane.

282

### 283 Statistical analysis

284 We performed statistical analyses using IBM SPSS Statistics 20.0 software. We first assessed the  
285 normality of each variable using the Shapiro Wilk test. As the data were not parametric, we determined  
286 the effects of state/time points on dependent variables using the one-factor Friedman test for each  
287 state/time points. When a main effect was found, we performed a Wilcoxon signed-rank test with

288 Bonferroni's correction. The critical level for a statistical significance was set at an  $\alpha$ -level of 0.05.  
289 Rayleigh's test was performed to determine whether phase intervals were randomly distributed, as  
290 described (Zar, 1974; Kjaerulff and Kiehn, 1996; Thibaudier and Frigon, 2014; Thibaudier et al., 2017;  
291 Audet et al., 2022). Briefly, we calculated the  $r$  value to measure the dispersion of phase interval values  
292 around the mean, with a value of 1 indicating a perfect concentration in one direction, and a value of 0  
293 indicating uniform dispersion. To test the significance of the directional mean, we performed Rayleigh's  
294  $z$  test:  $z = nr^2$ , where  $n$  is the sample size (number of steps). The  $z$  value was then compared to a critical  
295  $z$  value on Rayleigh's table to determine if there was a significant concentration around the mean ( $P$   
296 value).

297

## 298 **Results**

### 299 **The recovery of quadrupedal treadmill locomotion after staggered hemisections and extent of** 300 **spinal lesions**

301 In the present study, all eight cats spontaneously recovered quadrupedal treadmill locomotion at 0.4  
302 m/s one to two weeks following the first lateral hemisection at T5-T6 on the right side. All eight cats also  
303 recovered quadrupedal treadmill locomotion at 0.4 m/s one to four weeks following the second lateral  
304 hemisection at T10-T11 on the left side. **Figure 1** shows a schematic of the staggered hemisections  
305 and the extent of the first and second hemisections for each cat based on histological analysis, which  
306 ranged from 40.3% and 66.4% ( $50.1\% \pm 9.1$ ) and 33.5% and 53.7% ( $45.8\% \pm 6.5$ ) for the first and  
307 second lesions, respectively.

308 **Table 1** summarizes three features of locomotor performance after the first and second hemisections.  
309 After the first hemisection, only one cat (Cat AR) required balance assistance, where an experimenter  
310 held the tail to provide mediolateral balance but not weight support, and only at weeks 1-2. Cats did not  
311 require perineal stimulation to perform quadrupedal locomotion after the first hemisection. After the  
312 second hemisection, some cats did not recover quadrupedal locomotion until weeks 3 or 4 and all cats  
313 required balance assistance at both time points (weeks 1-4 and 7-8). It is important to note that holding  
314 the tail was not used for hindquarter weight support, only for balance assistance. After the second

315 hemisection, 5 of 8 and 3 of 8 cats required perineal stimulation at weeks 1-4 and 7-8, respectively. The  
 316 three cats requiring perineal stimulation at weeks 7-8 also needed it at weeks 1-4.

317 After the first hemisection on the right side of the spinal cord, all eight cats maintained left digitigrade  
 318 hindpaw placement (contralateral to the lesion). Most cats (6 out of 8) also retained right digitigrade  
 319 hindpaw placement (ipsilateral to the lesion). However, one cat (Cat PO) showed no digitigrade  
 320 placement of the right hindpaw at week 1 after the first hemisection, while another cat (Cat GR)  
 321 performed proper placement 70% of the time. In both cases, the cats placed the right hindpaw on its  
 322 dorsum. At weeks 7-8 after the first hemisection, all cats performed left and right digitigrade placement.  
 323 The second hemisection on the left side did not affect digitigrade placement of the right hindpaw in 7 of  
 324 8 cats. Only Cat MB showed impaired right hindpaw digitigrade placement with 71% and 48% at weeks  
 325 3 and 8, respectively. Surprisingly, most cats (5 out of 8) maintained left digitigrade hindpaw placement  
 326 at weeks 1-4 after the second hemisection on the left side. Cat MB did not recover left digitigrade  
 327 placement while cats GR and PO showed impaired left digitigrade placement at weeks 3-4 that  
 328 recovered at weeks 7-8 after the second hemisection.

329  
 330

**Table 1. Locomotor performance of individual cats after the first and second hemisections**

Cats	Time point	Balance assistance	Perineal stimulation required	Left digitigrade Paw placement	Right digitigrade Paw placement
TO	Hemi 1, wk 2	No	No	Yes	Yes
	Hemi 1, wk 8	No	No	Yes	Yes
	Hemi 2, wk 3	Yes	Yes	Yes	Yes
	Hemi 2, wk 7	Yes	No	Yes	Yes
JA	Hemi 1, wk 2	No	No	Yes	Yes
	Hemi 1, wk 8	No	No	Yes	Yes
	Hemi 2, wk 2	Yes	Yes	Yes	Yes
	Hemi 2, wk 7	Yes	No	Yes	Yes
AR	Hemi 1, wk 2	Yes	Yes	Yes	Yes
	Hemi 1, wk 8	No	No	Yes	Yes
	Hemi 2, wk 1	Yes	Yes	Yes	Yes
	Hemi 2, wk 7	Yes	Yes	Yes	Yes
HO	Hemi 1, wk 2	No	No	Yes	Yes
	Hemi 1, wk 8	No	No	Yes	Yes
	Hemi 2, wk 3	Yes	No	Yes	Yes
	Hemi 2, wk 7	Yes	No	Yes	Yes
MB	Hemi 1, wk 2	No	No	Yes	Yes
	Hemi 1, wk 7	No	No	Yes	Yes
	Hemi 2, wk 3	Yes	Yes	No	71%
	Hemi 2, wk 8	Yes	Yes	No	48%
GR	Hemi 1, wk 1	No	No	Yes	70%
	Hemi 1, wk 8	No	No	Yes	Yes
	Hemi 2, wk 3	Yes	No	57%	Yes
	Hemi 2, wk 8	Yes	No	Yes	Yes
KA	Hemi 1, wk 2	No	No	Yes	Yes
	Hemi 1, wk 8	No	No	Yes	Yes
	Hemi 2, wk 3	Yes	No	Yes	Yes
	Hemi 2, wk 7	Yes	No	Yes	Yes
PO	Hemi 1, wk 1	No	No	Yes	No

Hemi 1, wk 8	No	No	Yes	Yes
Hemi 2, wk 4	Yes	Yes	26%	Yes
Hemi 2, wk 7	Yes	Yes	Yes	Yes

331

332 **New patterns of forelimb-hindlimb coordination emerge after the first and second hemisections**

333 In the present study, all intact cats performed 1:1 fore-hind coordination in 100% of trials, indicating  
 334 an equal number of steps at the shoulder and hip girdles, as shown for a single cat in **Figure 2** (top  
 335 panel). However, at weeks 1-2 and 7-8 after the first hemisection, all cats showed 2:1 fore-hind  
 336 coordination with varying proportions ( $48.9\% \pm 35.4\%$ ). When this occurred, cycles with 2:1 and 1:1  
 337 fore-hind coordination were intermingled within the same locomotor episode (**Fig. 2**, middle panels) and  
 338 some cats only showed patterns of 2:1 fore-hind coordination, as shown previously in rats and cats  
 339 (Górska et al., 1990, 1996, 2013; Bem et al., 1995; Barrière et al., 2010; Alluin et al., 2011; Leszczyńska  
 340 et al., 2015; Thibaudier et al., 2017). Interestingly, at weeks 1-4 and 7-8 weeks after the second  
 341 hemisection, some cats displayed a decrease (Cats JA, MB, GR, KA, PO) in the proportion of 2:1 fore-  
 342 hind coordination while others showed an increase (Cats TO, AR). In cat AR, the proportion of 2:1  
 343 coordination increased considerably (**Fig. 2**, bottom panels). **Table 2** summarizes the proportion of 2:1  
 344 fore-hind coordination in each cat after both hemisections.

345

346 **Table 2. Proportion of 2:1 fore-hind coordination after the first and second hemisections**

	First hemisection		Second hemisection	
	Weeks 1-2	Weeks 7-8	Weeks 1-4	Weeks 7-8
TO	100% (14/14)	100% (16/16)	62% (8/13)	100% (12/12)
JA	58% (7/12)	58% (11/19)	100% (8/8)	96% (22/23)
AR	50% (4/8)	100% (21/21)	75% (18/24)	96% (23/24)
HO	25% (6/24)	20% (2/10)	71% (12/17)	71% (15/21)
MB	100% (22/22)	82% (28/34)	29% (4/14)	11% (2/19)
GR	10% (1/10)	19% (7/36)	52% (11/21)	50% (6/12)
KA	15% (4/26)	9% (2/23)	100% (21/21)	71% (12/17)
PO	33% (6/18)	13% (1/8)	89% (17/19)	88% (7/8)

347

348 **Interlimb coordination is weaker and more variable after staggered hemisections**

349 To determine how the first and second hemisections affected temporal interlimb coordination, we  
 350 measured phase intervals between six limb pairs. Values of  $0^\circ$  or  $360^\circ$  indicate a strict in-phase coupling  
 351 (pacing gait), while a value of  $180^\circ$  indicates a strict out-of-phase coupling. Previous studies in cats have  
 352 used values between  $270^\circ$  and  $90^\circ$  to denote an in-phase coupling and values between  $90^\circ$  and  $270^\circ$   
 353 for out-of-phase coupling (English and Lennard, 1982; Thibaudier and Frigon, 2014; Audet et al., 2022).

354 To assess the step-by-step consistency of forelimb-hindlimb coordination, we performed Rayleigh's test  
355 and calculated the  $r$  value, a measure of angular dispersion around the mean for the coupling between  
356 the right forelimb and right hindlimb (right homolateral coupling) during tied-belt quadrupedal treadmill  
357 locomotion at 0.4 m/s before (intact) and at weeks 1-4 and 7-8 after the first and second hemisections.  
358 When the  $r$  value is close to 1.0 and significant, it indicates that phase intervals are oriented in a specific  
359 direction. We only show this analysis for right homolateral coupling because cats maintained 1:1  
360 coordination between the left and right sides at shoulder (forelimb coupling) and hip (hindlimb coupling)  
361 girdles.

362 In the intact state, we only observed 1:1 fore-hind coordination and right homolateral couplings mainly  
363 at 40-80° (**Fig. 3**). At weeks 1-2 and 7-8 after the first hemisection, we found greater dispersal during  
364 1:1 coordination but most right homolateral couplings were from 0-90°. With 2:1 fore-hind coordination,  
365 right homolateral couplings were dispersed with the first and second forelimb steps mainly from 0-240°  
366 and 120-360°, respectively. After the second hemisection, right homolateral couplings remained  
367 dispersed with no clear preference with 1:1 coordination. With 2:1 coordination, right homolateral  
368 couplings resembled those found after the first hemisection.

369 **Table 3** shows  $r$  values from Rayleigh's test for phase intervals of right homolateral coupling for  
370 individual cats where we separated cycles with 1:1 and 2:1 fore-hind coordination. Note that some cats  
371 did not display 1:1 coordination after the first and/or second hemisections. All cats had 1:1 coordination  
372 in the intact state, with  $r$  values ranging from 0.79 to 1.00 (mean  $0.92 \pm 0.07$ ). All  $r$  values were  
373 significant, indicating consistent step-by-step fore-hind coordination. At weeks 1-2 after the first  
374 hemisection, six of eight cats had cycles with 1:1 coordination, with  $r$  values ranging from 0.45 to 0.94  
375 (mean  $0.78 \pm 0.21$ ). All  $r$  values were significant except for cat JA. At weeks 1-2 after the first  
376 hemisection, seven of eight cats had cycles with 2:1 coordination, with  $r$  values for the first forelimb step  
377 ranging from 0.12 to 0.85 (mean  $0.48 \pm 0.29$ ). Only cat TO had a significant  $r$  value. For the second  
378 forelimb step,  $r$  values ranged from 0.27 to 0.70 (mean  $0.54 \pm 0.15$ ) and three  $r$  values were significant  
379 and four were not. At weeks 7-8 after the first hemisection, six of eight cats had cycles with 1:1  
380 coordination, with  $r$  values ranging from 0.13 to 0.85 (mean  $0.61 \pm 0.26$ ). Four of six cats had significant



381 r values. Six of eight cats had cycles with 2:1 coordination, with r values ranging from 0.30 to 0.84 (mean  
382  $0.50 \pm 0.23$ ) and 0.12 to 0.80 (mean  $0.40 \pm 0.25$ ) for the first and second forelimb steps, respectively.  
383 Four and three of six cats had significant r vales for the first and second forelimb steps, respectively. At  
384 weeks 1-4 after the second hemisection, six of eight cats had cycles with 1:1 coordination, with r values  
385 ranging from 0.38 to 0.91 (mean  $0.62 \pm 0.22$ ). Three of six cats had significant r values. All eight cats  
386 had cycles with 2:1 coordination, with r values ranging from 0.39 to 0.83 (mean  $0.51 \pm 0.14$ ) and 0.12  
387 to 0.86 (mean  $0.49 \pm 0.21$ ) for the first and second forelimb steps, respectively. Three and four of eight  
388 cats had significant r vales for the first and second forelimb steps, respectively. At weeks 7-8 after the  
389 second hemisection, only four of eight cats had cycles with 1:1 coordination, with r values ranging from  
390 0.44 to 0.97 (mean  $0.72 \pm 0.24$ ). Three of four cats had significant r values. All eight cats had cycles  
391 with 2:1 coordination, with r values ranging from 0.35 to 0.70 (mean  $0.49 \pm 0.11$ ) and 0.10 to 0.64 (mean  
392  $0.43 \pm 0.17$ ) for the first and second forelimb steps, respectively. Three of eight cats had significant r  
393 vales for the first and second forelimb steps.

394 Therefore, based on r values and their significance (or lack thereof), as well as the distributions of  
395 phase intervals shown in **Figure 3**, fore-hind coordination weakens and becomes more variable after  
396 the first hemisection, even when separating cycles with 1:1 and 2:1 coordination. Surprisingly, the  
397 second hemisection on the left side had little additional effect on fore-hind coordination compared to  
398 what we observed after first hemisection.

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407 **Table 3. Circular statistics for forelimb-hindlimb coordination before and after staggered**  
 408 **hemisections.**

Cats	Time points	1:1 coordination	2:1 – 1 <sup>st</sup> step	2:1 – 2 <sup>nd</sup> step
		r	r	r
TO	Intact	0.79*	-	-
	Hemi 1, wk 2	-	0.74*	0.68*
	Hemi 1, wk 8	-	0.75*	0.44*
	Hemi 2, wk 3	0.91*	0.42	0.60*
	Hemi 2, wk 7	-	0.70*	0.64*
JA	Intact	0.86*	-	-
	Hemi 1, wk 2	0.45	0.64	0.27
	Hemi 1, wk 8	0.74*	0.30	0.35
	Hemi 2, wk 2	-	0.83*	0.86*
	Hemi 2, wk 6	-	0.58*	0.33
AR	Intact	0.88*	-	-
	Hemi 1, wk 2	0.94*	0.85	0.49
	Hemi 1, wk 8	-	0.84*	0.80*
	Hemi 2, wk 1	0.80*	0.47*	0.54*
	Hemi 2, wk 7	-	0.51*	0.57*
HO	Intact	0.97*	-	-
	Hemi 1, wk 2	0.57*	0.23	0.48
	Hemi 1, wk 8	0.69*	-	-
	Hemi 2, wk 3	0.44	0.46	0.48
	Hemi 2, wk 7	0.87*	0.42	0.42
MB	Intact	0.96*	-	-
	Hemi 1, wk 2	-	0.23	0.54*
	Hemi 1, wk 7	0.52	0.33	0.50*
	Hemi 2, wk 3	0.71*	0.39	0.49
	Hemi 2, wk 8	0.60*	0.48	0.10
GR	Intact	1.00*	-	-
	Hemi 1, wk 1	0.92*	-	-
	Hemi 1, wk 8	0.75*	0.37	0.17
	Hemi 2, wk 3	0.38	0.61*	0.12
	Hemi 2, wk 8	0.44	0.41	0.39
KA	Intact	0.97*	-	-
	Hemi 1, wk 2	0.93*	0.12	0.64
	Hemi 1, wk 8	0.85*	0.42	0.12
	Hemi 2, wk 3	-	0.46*	0.38
	Hemi 2, wk 7	0.97*	0.48	0.57*
PO	Intact	0.90*	-	-
	Hemi 1, wk 1	0.88*	0.55	0.70*
	Hemi 1, wk 8	0.13	-	-
	Hemi 2, wk 4	0.46	0.46*	0.47*
	Hemi 2, wk 7	-	0.35	0.43

409  
 410 To determine how staggered hemisections affected the coordination between limbs of the same  
 411 girdle, we measured phase intervals for forelimb and hindlimb couplings (**Fig. 4A**). For these analyses,  
 412 we pooled cycles with 1:1 and 2:1 fore-hind coordination because some cats did not show 1:1  
 413 coordination after the first and/or second hemisections. A decrease or an increase in phase interval  
 414 indicates that the left limb made contact earlier or later, respectively, in the normalized cycle relative to  
 415 the right limb. For forelimb coupling, we found a significant decrease in the phase interval at weeks 1-4  
 416 and 7-8 after the second hemisection compared to the intact state, indicating earlier contact of the left  
 417 forelimb relative to the right forelimb. For hindlimb coupling, we found a significant increase in the phase

418 interval at weeks 1-4 and 7-8 after the second hemisection compared to weeks 1-2 and 7-8 after the  
419 first hemisection, indicating delayed contact of the left hindlimb relative to the right hindlimb.

420 To determine if cats displayed greater variations in limb couplings after staggered hemisection, we  
421 measured coefficients of variation for all six limb pairs (**Fig. 4B**). We found a significant main effect of  
422 state for all limb couplings except for forelimb coupling. Thus, forelimb coupling remains consistent on  
423 a step-by-step basis after hemisections. For hindlimb coupling, the coefficient of variation was  
424 significantly greater at weeks 7-8 weeks after the second hemisection compared to weeks 1-2 after the  
425 first hemisection only. The pattern of change in coefficients of variations for homolateral and diagonal  
426 couplings is more revealing. After the first hemisection, we observed a significant increase in the  
427 coefficient of variations for left homolateral coupling at both weeks 1-2 and 7-8 compared to the intact  
428 state but after the second hemisection, no significant differences with the intact state were found. For  
429 right homolateral coupling, the coefficient of variation was significantly greater at weeks 7-8 after the  
430 first hemisection compared to the intact state only. Left and right diagonal couplings on the other hand  
431 showed greater coefficients of variations after the second hemisection at both weeks 1-4 and 7-8  
432 compared to the intact state. Thus, when considering fore-hind coordination, the second hemisection  
433 had no significant additional effect on variability compared to the first hemisection.

434

### 435 **Staggered hemisections generate temporal adjustments in the fore- and hindlimbs and reversals** 436 **of left-right asymmetries in the hindlimbs**

437 To determine temporal adjustments of the fore- and hindlimbs during quadrupedal treadmill  
438 locomotion, we measured cycle and phase durations before and after the two hemisections. For these  
439 measurements, we pooled cycles with 1:1 and 2:1 fore-hind coordination because some cats did not  
440 show 1:1 coordination after the first and/or second hemisections. For the forelimbs (**Fig. 5A**), we  
441 observed a significant reduction in LF and RF cycle and stance durations after the second hemisection  
442 at weeks 1-2 and 7-8 compared to the intact state and at weeks 1-4 after the second hemisection  
443 compared to weeks 7-8 after the first (**Fig. 5A**). Compared to the intact state, LF and RF swing durations  
444 were significantly reduced at weeks 1-4 and 7-8 after the second hemisection, and at weeks 1-4 after

445 the second hemisection compared to weeks 7-8 after the first for LF. Changes in forelimb cycle and  
446 phase durations are undoubtedly due to the appearance of 2:1 fore-hind coordination.

447 For the hindlimbs (**Fig. 5B**), we observed no significant change in LH cycle duration after staggered  
448 hemisections, but we found a main effect for RH cycle duration with an increase at weeks 7-8 after the  
449 second hemisection compared to the intact state. LH stance duration did not change significantly  
450 compared to the intact state after staggered hemisections, but we did observe a significant decrease at  
451 weeks 1-4 after the second hemisection compared to weeks 1-2 and 7-8 after the first. RH stance  
452 duration did not change significantly after staggered hemisections. LH swing duration was longer at  
453 weeks 1-4 after the second hemisection compared to the intact state and weeks 1-2 after the first  
454 hemisection. RH swing duration was longer at weeks 1-2 and 7-8 after the first hemisection and at  
455 weeks 7-8 after the second hemisection compared to the intact state.

456 To determine if staggered hemisections produced left-right asymmetries in cycle and phase durations  
457 at shoulder and hip girdles, we measured an asymmetry index by subtracting right limb durations from  
458 left limb durations (**Fig. 5C**). We found no significant asymmetries in the forelimbs. However, for the  
459 hindlimbs, while we observed no asymmetries in cycle duration (cats maintained 1:1 coordination  
460 between hindlimbs), stance and swing durations displayed marked asymmetries after the first and  
461 second hemisections. The asymmetry index for hindlimb stance duration became negative after the first  
462 hemisection, with longer LH stance duration, before switching to positive after the second hemisection,  
463 with longer RH stance duration. The asymmetry index for hindlimb swing durations showed an opposite  
464 pattern, becoming positive and negative after the first and second hemisections, respectively, indicating  
465 that LH swing duration is shorter and longer than RH swing duration after the first and second  
466 hemisections, respectively.

467

#### 468 **Cats adjust their support periods after staggered hemisections during quadrupedal locomotion**

469 We generally find eight individual support periods during quadrupedal locomotion in a normalized  
470 cycle (Frigon et al., 2014; Lecomte et al., 2022). However, a period of double support can become a  
471 period of quadrupedal support in some cycles, thus we can find nine different support periods. The

472 proportion of some support periods significantly increased after spinal hemisections, while others  
473 decreased (**Fig. 6**). For example, the two periods of triple support involving both hindlimbs (Periods 1  
474 and 5) decreased after the two hemisections compared to the intact state, except at weeks 7-8 after the  
475 first hemisection. Periods of diagonal support (Periods 2 and 6) increased after the second hemisection  
476 compared to the intact state. Period 2, involving the left forelimb and right hindlimb, increased  
477 significantly at weeks 1-4 and 7-8 after the second hemisection compared to the intact state and at  
478 weeks 1-4 after the second compared to weeks 1-2 and 7-8 after the first. Period 6 increased at weeks  
479 1-4 and 7-8 after the second hemisection compared to the intact state. The triple support period involving  
480 the two forelimbs and the right hindlimb (Period 3) increased after the second hemisection at weeks 1-  
481 4 and 7-8 compared to weeks 1-2 and 7-8 after the first. Left homolateral double support (Period 8) did  
482 not change significantly after staggered hemisections compared to the intact state. However, it was  
483 significantly shorter at weeks 1-4 after the second hemisection compared to both time points after the  
484 first hemisection and at weeks 7-8 after the second compared to weeks 7-8 after the first. We observed  
485 no significant changes after staggered hemisections for right homolateral support (Period 4), the triple  
486 support period involving the left hindlimb and both forelimbs (Period 7) and quadrupedal support (Period  
487 9). Therefore, cats adjust their support periods to maintain dynamic balance during quadrupedal  
488 locomotion after staggered hemisections, initially favoring support away from the right hindlimb (side of  
489 first hemisection) and then away from the left hindlimb (side of second hemisection).

490

### 491 **Staggered hemisections generate spatial adjustments in the fore- and hindlimbs but few left-** 492 **right spatial asymmetries in the hindlimbs**

493 To determine how staggered hemisections affected spatial parameters, we measured stride length,  
494 the horizontal distance traveled by each limb from contact to contact and the horizontal distance of the  
495 fore- and hindpaws from the shoulder and hip, respectively, at contact and liftoff. Compared to the intact  
496 state, forelimb stride lengths decreased bilaterally but only after the second hemisection, consistent with  
497 smaller steps with 2:1 fore-hind coordination (**Fig. 7A**). We observed that the distance of RF relative to  
498 the shoulder at liftoff was more rostral at weeks 1-4 after the second hemisection compared to the intact

499 state while LF positioning did not change. Forelimb placement at contact relative to the shoulder did not  
500 change significantly.

501 Hindlimb stride length did not significantly change after staggered hemisections for LH and although  
502 RH showed a significant main effect, we observed no significant difference between time points (**Fig.**  
503 **7B**). However, we observed several changes in the position of the hindpaw relative to the hip. We found  
504 a more caudal horizontal distance between the left hindpaw and the hip at liftoff at both time points after  
505 the second hemisection compared to the intact state. Similarly, we found a more caudal horizontal  
506 distance between the right hindpaw and the hip at liftoff at both time points after the second hemisection  
507 compared to the intact state and at weeks 7-8 after the first hemisection. The right and left hindpaw  
508 were closer to the hip at contact at weeks 1-4 after the second hemisection compared to the intact state  
509 and weeks 7-8 after the first hemisection.

510 To determine if staggered hemisections produced asymmetric changes in spatial variables between  
511 the left and right sides at shoulder and hip girdles, we measured an asymmetry index by subtracting  
512 right limb values from left limb values (**Fig. 7C**). For the forelimbs, we found no significant asymmetries.  
513 For the hindlimbs, we found a significant main effect for stride length but pairwise comparisons revealed  
514 no differences between time points. For the distance at contact, we only observed a significant difference  
515 between weeks 1-2 after the first hemisection and weeks 1-4 after the second hemisection, where left  
516 and right placements were more rostral relative to the hip after the first and second hemisections,  
517 respectively.

518

### 519 **Forelimb movements adjust to avoid interference after staggered hemisections**

520 To assess limb interference, we measured the horizontal distance between the toe markers of the  
521 fore- and hindlimbs at contact and liftoff of the left and right forelimbs (**Fig. 8**), as described previously  
522 in spinal cats during quadrupedal locomotion (Audet et al., 2022). The left distance, the distance  
523 between LF and LH toe markers, increased at LF contact at weeks 1-4 and 7-8 after the second  
524 hemisection compared to the intact state and weeks 7-8 after the first hemisection. At LF liftoff, the left  
525 distance increased at weeks 1-4 and 7-8 after the second hemisection compared to the intact state. The

526 right distance, the distance between RF and RH toe markers, increased at weeks 1-2 after the first  
527 hemisection and at weeks 1-4 and 7-8 after the second hemisection at both RF contact and liftoff. We  
528 propose that increased distances between the fore- and hindlimbs helps avoid interference between the  
529 fore- and hindlimbs.

530

### 531 **The recovery of hindlimb locomotion after staggered hemisections is mediated by a spinal** 532 **mechanism**

533 As stated in the introduction, a spinal mechanism plays a prominent role in the recovery of hindlimb  
534 locomotion following an incomplete SCI (Barrière et al., 2008). To determine if a spinal mechanism also  
535 contributes to hindlimb locomotor recovery after staggered hemisections, we performed a spinal  
536 transection at T12-T13 nine to ten weeks after the second hemisection in three cats (TO, HO, JA). In all  
537 three cats, hindlimb locomotion was expressed the day following transection, a recovery that normally  
538 takes a minimum of three weeks (Lovely et al., 1986; Barbeau and Rossignol, 1987; Barrière et al.,  
539 2008; Harnie et al., 2019). **Figure 9A** shows a representative example from one cat before transection  
540 (i.e. data collected at week 7 after the second hemisection) and at days 1, 2 and 7 after transection  
541 without (top panel) and with (bottom panel) perineal stimulation. We can see EMG activity in selected  
542 hindlimb muscles during hindlimb-only locomotion. Cat JA stepped one day after the transection without  
543 and with perineal stimulation. On the second day, however, the hindlimbs dragged on the treadmill  
544 without perineal stimulation but the pattern was robust with perineal stimulation. One week after  
545 transection, hindlimb-only locomotion was robust without and with perineal stimulation.

546 **Table 4** summarizes three features of locomotor performance before (pre-transection) and at days 1  
547 and 2 as well as weeks 1, 2 after transection without perineal stimulation. Pre-transection, all three cats  
548 performed left and right digitigrade paw placement without perineal stimulation but required balance  
549 assistance. One day after transection, two cats (TO and HO) did not perform left and right digitigrade  
550 paw placement, while Cat JA performed left digitigrade paw placement and right digitigrade paw  
551 placement 80% of the time. With the addition of perineal stimulation all three cats performed left and  
552 right digitigrade paw placement, but still required balance assistance. Two days after transection, Cats

553 TO performed proper digitigrade placement bilaterally without and with perineal stimulation. In contrast,  
 554 for Cats JA and HO, perineal stimulation was required to perform proper placement bilaterally. One and  
 555 two weeks after transection, all three cats performed left and right digitigrade paw placement without  
 556 and with perineal stimulation, but still required balance assistance. Three weeks after transection, Cat  
 557 TO only performed proper left digitigrade paw placement 57% of the time without perineal stimulation.  
 558 In contrast, the two other cats performed proper left and right digitigrade paw placement without and  
 559 with perineal stimulation.

560

561 **Table 4. Locomotor performance of individual cats before and after spinal transection during**  
 562 **hindlimb-only treadmill locomotion without perineal stimulation**

Cats	Time point	Balance assistance	Left digitigrade Paw placement	Right digitigrade Paw placement
TO	Pre spinal	Yes	Yes	Yes
	Spinal, day 1	Yes	No	No
	Spinal, day 2	Yes	Yes	Yes
	Spinal, wk 1	Yes	Yes	Yes
	Spinal, wk 2	Yes	Yes	Yes
	Spinal, wk 3	Yes	57%	Yes
JA	Pre spinal	Yes	Yes	Yes
	Spinal, day 1	Yes	Yes	80%
	Spinal, day 2	Yes	No	No
	Spinal, wk 1	Yes	Yes	Yes
	Spinal, wk 2	Yes	Yes	Yes
	Spinal, wk 3	Yes	Yes	Yes
HO	Pre spinal	Yes	Yes	Yes
	Spinal, day 1	Yes	No	No
	Spinal, day 2	Yes	No	No
	Spinal, wk 1	Yes	Yes	Yes
	Spinal, wk 2	Yes	Yes	Yes
	Spinal, wk 3	Yes	Yes	Yes

563

564 We and others have shown that cats can perform quadrupedal locomotion after a complete thoracic  
 565 spinal transection (Shurrager and Dykman, 1951; Eidelberg et al., 1980; Howland et al., 1995; Audet et  
 566 al., 2022). In the present study, all three cats performed quadrupedal treadmill locomotion at 0.4 m/s  
 567 with perineal stimulation and balance assistance after spinal transection. Instead of 1:1 and 2:1 fore-  
 568 hind coordination patterns observed at weeks 7-8 after the second hemisection, we observed a 1:2 fore-  
 569 hind coordination in some cycles, indicating that one hindlimb could take two steps within a single  
 570 forelimb cycle (**Fig. 9B**), as shown recently in spinal-transected cats (Audet et al., 2022). In all three  
 571 cats, cycles with 1:2 fore-hind coordination were interspersed with 1:1 coordination. The 1:2 fore-hind



572 coordination represented 21% (Cat TO), 20% (Cat JA) and 48% (Cat HO) of cycles. It is possible that  
573 perineal stimulation played a role in the emergence of 1:2 coordination.

574

## 575 **Discussion**

576 We showed that cats spontaneously recovered quadrupedal locomotion following staggered  
577 hemisections but required balance assistance after the second. We hypothesized that the second  
578 hemisection would more greatly disrupt fore-hind coordination. However, the first hemisection weakened  
579 fore-hind coordination and made it more variable, with little additional effect of the second hemisection.  
580 Consistent with our hypothesis, hindlimb locomotion was expressed the day after spinal transection in  
581 cats that had recovered following the second hemisection. Below we discuss adjustments in the pattern  
582 and potential neuroplastic changes that allowed cats to maintain and recover some level of quadrupedal  
583 locomotor functionality.

584

### 585 ***Recovery of posture and locomotion after staggered hemisections***

586 Lesion extent varied between animals (**Fig. 1**). Generally, smaller lesions associate with faster and  
587 more complete locomotor recovery (Barrière et al., 2008; Rossignol et al., 2009). At weeks 1-2 after the  
588 first hemisection, only one cat required balance assistance (**Table 1**) while at weeks 7-8, no cat required  
589 balance assistance. After the second hemisection, all cats required balance assistance at both time  
590 points. Although hindquarter weight support was present in all cats after both hemisections, maintaining  
591 posture was challenging after the second. Weight support can be controlled at a spinal level whereas  
592 postural control requires supraspinal inputs (Macpherson et al., 1997). Thus, remaining pathways  
593 transmitting signals from supraspinal structures and potentially new ones bridging the lesions, such as  
594 short propriospinal pathways, are insufficient to restore postural control.

595 Although all cats recovered quadrupedal locomotion after staggered hemisections, some cats  
596 required perineal stimulation after the second hemisection (**Table 1**), which increases spinal neuronal  
597 excitability and facilitates hindlimb locomotion in spinal mammals through an undefined mechanism  
598 (Eidelberg et al., 1980; Alluin et al., 2015; Harnie et al., 2019; Merlet et al., 2021; Audet et al., 2022).



599 Previous studies proposed that the amount of locomotor training constitutes an important factor in  
600 locomotor recovery after partial spinal lesions (Kloos et al., 2005; Rossignol et al., 2009). We recently  
601 showed that hindlimb locomotor recovery in spinal cats occurs largely spontaneously without task-  
602 specific training (Harnie et al., 2019). Here, although cats did not receive treadmill training after  
603 staggered hemisections, they performed various tasks that can be considered training (see Methods).  
604 Cats were also freely moved in their cage and in a dedicated room. They could have developed  
605 compensatory behavioral strategies through self-training and some cats are naturally more active and  
606 athletic than others.

607

### 608 ***Interlimb coordination is different, weaker and more variable***

609 We observed 2:1 fore-hind coordination after the first and second hemisections, as shown previously  
610 (Eidelberg et al., 1980; Kato et al., 1984; Howland et al., 1995; Jiang and Drew, 1996; Brustein and  
611 Rossignol, 1998; Barrière et al., 2010; Alluin et al., 2011; Górska et al., 2013; Thibaudier et al., 2017).  
612 Intact cats also perform 2:1 fore-hind coordination on a transverse split-belt treadmill when forelimbs  
613 step faster than the hindlimbs (Thibaudier et al., 2013; Thibaudier and Frigon, 2014). This led to the  
614 hypothesis that forelimb CPGs have an intrinsically faster rhythmicity than hindlimb CPGs (Thibaudier  
615 et al., 2017), which is supported by findings in neonatal rats (Juvin et al., 2005). The 2:1 fore-hind  
616 coordination after incomplete SCI could result from reduced inhibition from hindlimb to forelimb CPGs  
617 (Górska et al., 2013; Frigon, 2017; Thibaudier et al., 2017), whereby reduced inhibition following thoracic  
618 SCI releases the intrinsically faster rhythmicity of forelimb CPGs. Disrupting serotonergic spinal  
619 pathways in intact rats also produces 2:1 fore-hind coordination (Sławińska *et al.* 2021). Functionally,  
620 2:1 coordination could represent a strategy to maximize static and dynamic stability (Thibaudier et al.,  
621 2017). Performing smaller steps keeps the center of gravity within the support polygon (Cartmill et al.,  
622 2002). Another functional reason could be to avoid interference of fore- and hindlimbs (**Fig. 8**). To avoid  
623 interference, cats often adopt pacing on a treadmill where homolateral limbs move in phase (Błaszczuk  
624 and Loeb, 1993). However, after incomplete SCI, cats might not be able to transition to a pacing gait.

625 We showed weaker and more variable fore-hind coordination after staggered hemisections (**Figs. 3**  
626 **and 4**), consistent with previous studies in rats and cats (Kato et al., 1984; Stelzner and Cullen, 1991;  
627 Murray et al., 2010; Cowley et al., 2015). The second hemisection did not produce significant additional  
628 effects in terms of step-by-step consistency of fore-hind coordination. However, it is important to note  
629 that cats required balance assistance after the second hemisection and providing this aid undoubtedly  
630 facilitated fore-hind coordination. Impaired coordination between the fore- and hindlimbs could be due  
631 to lesioned propriospinal pathways between cervical and lumbar levels and direct supraspinal pathways  
632 to the lumbar cord (Sherrington and Laslett, 1903; English, 1980; Kato et al., 1984; Bareyre et al., 2004;  
633 Courtine et al., 2008). The loss of interlimb reflex pathways also could have contributed to impaired fore-  
634 hind coordination (Hurteau et al., 2018). (Frigon, 2017) argued that fore-hind coordination requires  
635 supraspinal commands.

636 Support periods reorganized after staggered hemisection (**Fig. 6**). Periods of triple support involving  
637 the two hindlimbs decreased after the first hemisection and remained decreased after the second.  
638 Periods of triple support involving the right hindlimb and both forelimbs, and both diagonal support  
639 periods increased after the second hemisection. The cat is most unstable in diagonal support, but these  
640 phases help propel the body forward, increasing quadrupedal locomotion efficiency (Farrell et al., 2014).  
641 When both forelimbs contact the ground, they provide greater stability. Thus, increased diagonal support  
642 and triple support involving the forelimbs could be a strategy to facilitate forward movement while  
643 maintaining stability after staggered hemisections.

644

### 645 ***Spinal sensorimotor circuits play a prominent role in hindlimb locomotor recovery***

646 Many mammals recover hindlimb locomotion after complete spinal transection because the spinal  
647 locomotor CPG can still interact with sensory feedback from the hindlimbs (Shurrager and Dykman,  
648 1951; Lovely et al., 1986, 1990; Barbeau and Rossignol, 1987; Bélanger et al., 1996; De Leon et al.,  
649 1998, 1999; Leblond et al., 2003; Cha et al., 2007; Harnie et al., 2019). (Barrière et al., 2008) also  
650 showed that the spinal locomotor CPG makes an important contribution to hindlimb locomotor recovery  
651 following incomplete SCI. Here, we extend these results by showing that hindlimb locomotion was

652 expressed the day following a spinal transection made 9-10 weeks after the second hemisection (**Fig.**  
653 **9**). This indicates that the spinal network controlling the hindlimbs had already undergone plastic  
654 changes after staggered hemisections, making it more independent from descending signals originating  
655 above the lesions. Changes in the spinal cord can include intrinsic changes in neuronal excitability  
656 (Murray et al., 2010) and/or in sensorimotor interactions from peripheral afferents (Frigon et al., 2009;  
657 Gossard et al., 2015). (Kato et al., 1984) observed that hindlimb movements were initiated following  
658 forward movement induced by the forelimbs after staggered hemisections, much like a pantomime  
659 horse. Signals from muscle and/or cutaneous afferents likely play a major role in initiating hindlimb  
660 movements after staggered hemisections. This is not to say that descending signals cannot still  
661 influence and control the lumbar CPG through new short relay propriospinal pathways (Cowley et al.,  
662 2015).

663

#### 664 ***Locomotor recovery involves a series of neuroplastic changes***

665 As mentioned above, we observed several changes in the locomotor pattern. **Figure 10**  
666 schematically illustrates potential changes in spinal sensorimotor circuits after staggered hemisections  
667 involved in locomotor recovery based on left-right asymmetries in cycle and phase durations (**Fig. 5**)  
668 and the immediate expression of hindlimb locomotion after spinal transection. After the first hemisection,  
669 ipsilesional lumbar neurons have weaker activity and longer stance phases and increased weight  
670 support of the left hindlimb increases load feedback from extensors and cutaneous afferents. The left  
671 spinal network increases its influence on the right spinal network. Anatomical and functional asymmetric  
672 changes take place within the spinal cord (Murray and Goldberger, 1974; Hultborn and Malmsten, 1983;  
673 Helgren and Goldberger, 1993; Frigon et al., 2009). New descending and ascending pathways also form  
674 to facilitate descending commands from and to the brain (Fouad et al., 2000; Raineteau et al., 2002;  
675 Ballermann and Fouad, 2006; Courtine et al., 2008; Ghosh et al., 2010; Rosenzweig et al., 2010).  
676 However, these are insufficient to restore fore-hind coordination. After the second hemisection, neurons  
677 of the right spinal network have recovered their activity and stance and weight support is  
678 longer/increased for the right hindlimb. The right spinal network increases its influence on the left one.

679 Direct ascending and descending pathways are disrupted but new pathways can form through short  
680 propriospinal relays (Zaporozhets et al., 2006; Cowley et al., 2008). However, these are insufficient to  
681 restore postural control. Over time after the second hemisection, spinal neuronal activity controlling the  
682 left hindlimb recovers. After spinal transection, both left and right spinal networks function without  
683 descending inputs and hindlimb locomotion is expressed, possibly via strengthened sensorimotor  
684 interactions bilaterally.

685

### 686 **Concluding remarks**

687 Staggered hemisections constitute an interesting SCI paradigm to investigate the recovery of  
688 posture, interlimb coordination and locomotion. We are currently investigating interlimb reflexes after  
689 staggered hemisections and their contribution to postural and locomotor recovery. Future studies need  
690 to determine what ascending and descending signals can be transmitted through such lesions, and  
691 importantly, if they make meaningful contributions to locomotion and how we can facilitate them using  
692 therapeutic approaches.

693

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950 **Table and figure legends**

951 **Table 1. Locomotor performance of individual cats after the first and second hemisections.**

952 Locomotor performance of eight cats using four criteria. Percent values indicate the percentage of steps  
953 with correct digitigrade placement.

954

955 **Table 2. Proportion of 2:1 fore-hind coordination after the first and second hemisections.** Percent

956 values indicate the percentage of cycles with 2:1 fore-hind coordination while the number in brackets  
957 indicate the number of cycles with 2:1 fore-hind coordination divided by the total number of hindlimb cycles  
958 recorded.

959

960 **Table 3. Circular statistics for forelimb-hindlimb coordination before and after staggered**

961 **hemisections.** The table shows r values from Rayleigh's test at the different time points for individual  
962 cats before and after hemisections for cycles with 1:1 and 2:1 (first and second forelimb steps)  
963 coordination. Asterisks indicate a significant r value. .

964

965 **Table 4. Locomotor performance of individual cats before and after spinal transection during**

966 **hindlimb-only treadmill locomotion without perineal stimulation.** Locomotor performance of three  
967 cats using four criteria. Percent values indicate the percentage of steps with correct digitigrade  
968 placement.

969

970 **Figure 1. Staggered hemisections paradigm and extent of lesions.** Schematic representation of the

971 staggered hemisections and extent of the first and second spinal lesions on the right (T5-T6) and left  
972 (T10-T11) sides, respectively, for individual cats. The black area represents the lesioned region.

973

974 **Figure 2. Quadrupedal treadmill locomotion before and after staggered hemisections.** Activity

975 from selected fore- (FL) and hindlimb (HL) muscles and stance phases (thick horizontal lines of the left

976 (L) and right (R) limbs in Cat AR at 0.4 m/s. Grey stance phases indicate cycles with 2:1 fore-hind  
977 coordination. BB, Biceps brachii; TRI, Triceps brachii; ECU, Extensor carpi ulnaris; SRT, Sartorius; SOL;  
978 Soleus.

979

980 **Figure 3. Coordination between right homolateral limbs before and after staggered hemisection.**

981 Distribution of right homolateral couplings for the group during 1:1 and 2:1 (first and second forelimb  
982 steps) fore-hind coordination. Each bar represents the number of right homolateral couplings found for  
983 all eight cats at phase intervals of ten degrees.

984

985 **Figure 4. Interlimb phasing and variations during quadrupedal treadmill locomotion before and**

986 **after staggered hemisections for the group.** A) Phase intervals for forelimb and hindlimb couplings.

987 B) Coefficients of variation for six limb pairs. We averaged 8-36 cycles per cat at each time point. The  
988 bars represent mean  $\pm$  SD for the group ( $n = 8$  cats) while grey circles represent individual data points  
989 (mean for each cat). The  $P$  values show the main effect of state (one-factor Friedman test). Asterisks  
990 indicate significant differences between time points from the Wilcoxon signed-rank test with Bonferroni's  
991 correction.

992

993 **Figure 5. Temporal adjustments during quadrupedal treadmill locomotion before and after**

994 **staggered hemisections for the group.** A and B) Cycle, stance and swing durations for the fore- and

995 hindlimbs, respectively. C) Asymmetry indexes of temporal variables. We averaged 8-36 cycles per cat.

996 The bars represent mean  $\pm$  SD for the group ( $n = 8$  cats) while grey circles represent individual data  
997 points (mean for each cat). The  $P$  values show the main effect of state (one-factor Friedman test).  
998 Asterisks indicate significant differences between time points from the Wilcoxon signed-rank test with  
999 Bonferroni's correction.

1000

1001 **Figure 6. Support periods during quadrupedal treadmill locomotion before and after staggered**

1002 **hemisection for the group.** Individual periods of support normalized to right hindlimb cycle duration.

1003 We averaged 8-36 cycles per cat. The bars represent mean  $\pm$  SD for the group ( $n = 8$  cats) while grey  
1004 circles represent individual data points (mean for each cat). The  $P$  values show the main effect of state  
1005 (one-factor Friedman test). Asterisks indicate significant differences between time points from the  
1006 Wilcoxon signed-rank test with Bonferroni's correction.

1007

1008 **Figure 7. Spatial adjustments during quadrupedal treadmill locomotion before and after**  
1009 **staggered hemisections for the group.** A and B) Stride length and distances at contact and liftoff for  
1010 the fore- and hindlimbs, respectively. C) Asymmetry indexes of spatial variables. We averaged 8-36  
1011 cycles per cat. The bars represent mean  $\pm$  SD for the group ( $n = 8$  cats) while grey circles represent  
1012 individual data points (mean for each cat). The  $P$  values show the main effect of state (one-factor  
1013 Friedman test). Asterisks indicate significant differences between time points from the Wilcoxon signed-  
1014 rank test with Bonferroni's correction.

1015

1016 **Figure 8. Homolateral limb interference during quadrupedal treadmill locomotion before and**  
1017 **after staggered hemisections for the group.** Each panel shown horizontal distances between  
1018 homolateral hindlimbs (HL) and forelimbs (FL) at contact and liftoff of the left and right forelimb. We  
1019 averaged 8-36 ( $17.94 \pm 7.08$ ) cycles per cat. The bars represent mean  $\pm$  SD for the group ( $n = 8$  cats)  
1020 while grey circles represent individual data points (mean for each cat). The  $P$  values show the main  
1021 effect of state (one-factor Friedman test). Asterisks indicate significant differences between time points  
1022 from the Wilcoxon signed-rank test with Bonferroni's correction.

1023

1024 **Figure 9. Hindlimb-only and quadrupedal treadmill locomotion before and after complete spinal**  
1025 **transection.** A) Activity from selected hindlimb muscles and stance phases (thick horizontal lines of the  
1026 left (LHL) and right (RHL) hindlimbs in Cat JA at 0.4 m/s. B) Activity from selected hindlimb muscles and  
1027 stance phases (thick horizontal lines of the left (L) and right (R) limbs in Cat HO at 0.4 m/s. Grey and  
1028 blue stance phases indicate cycles with 2:1 and 1:2 fore-hind coordination, respectively. BB, Biceps  
1029 brachii; SOL; Soleus; SRT, Sartorius; Triceps brachii.



1030

1031 **Figure 10. Potential changes in spinal sensorimotor circuits after staggered hemisections.** In the  
1032 intact state, descending supraspinal and propriospinal pathways reach lumbar spinal interneurons that  
1033 control spinal motoneurons. Pathways transmitting signals from proprioceptive and cutaneous afferents  
1034 ascend to the brain and project locally to spinal interneurons. After the first hemisection performed on  
1035 the right side, ipsilesional lumbar neurons have weaker activity and increased weight support of the  
1036 contralesional hindlimb increases load feedback from extensors and cutaneous afferents. Thicker lines  
1037 represent increase influence. The left spinal network increases its influence on the right spinal network.  
1038 New descending and ascending pathways also form to facilitate communication between the brain and  
1039 spinal cord. After the second hemisection performed on the left side, neurons of the right spinal network  
1040 have recovered their activity. Direct ascending and descending pathways are disrupted but new  
1041 pathways can form through short propriospinal relays. After spinal transection, both the left and right  
1042 spinal network function without descending inputs and hindlimb locomotion is expressed, possibly via  
1043 strengthened sensorimotor interactions bilaterally.

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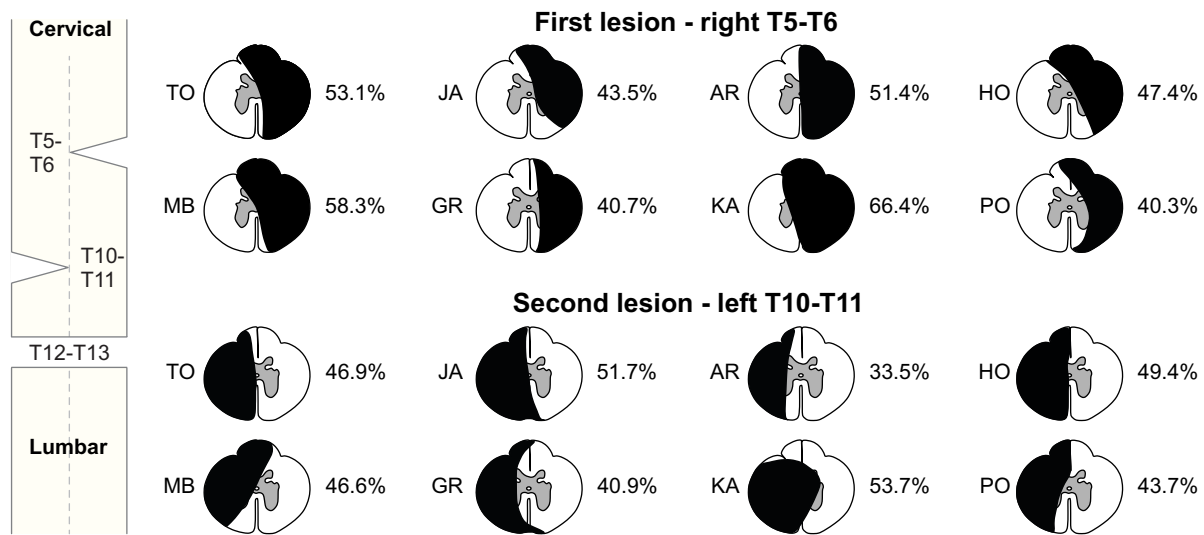


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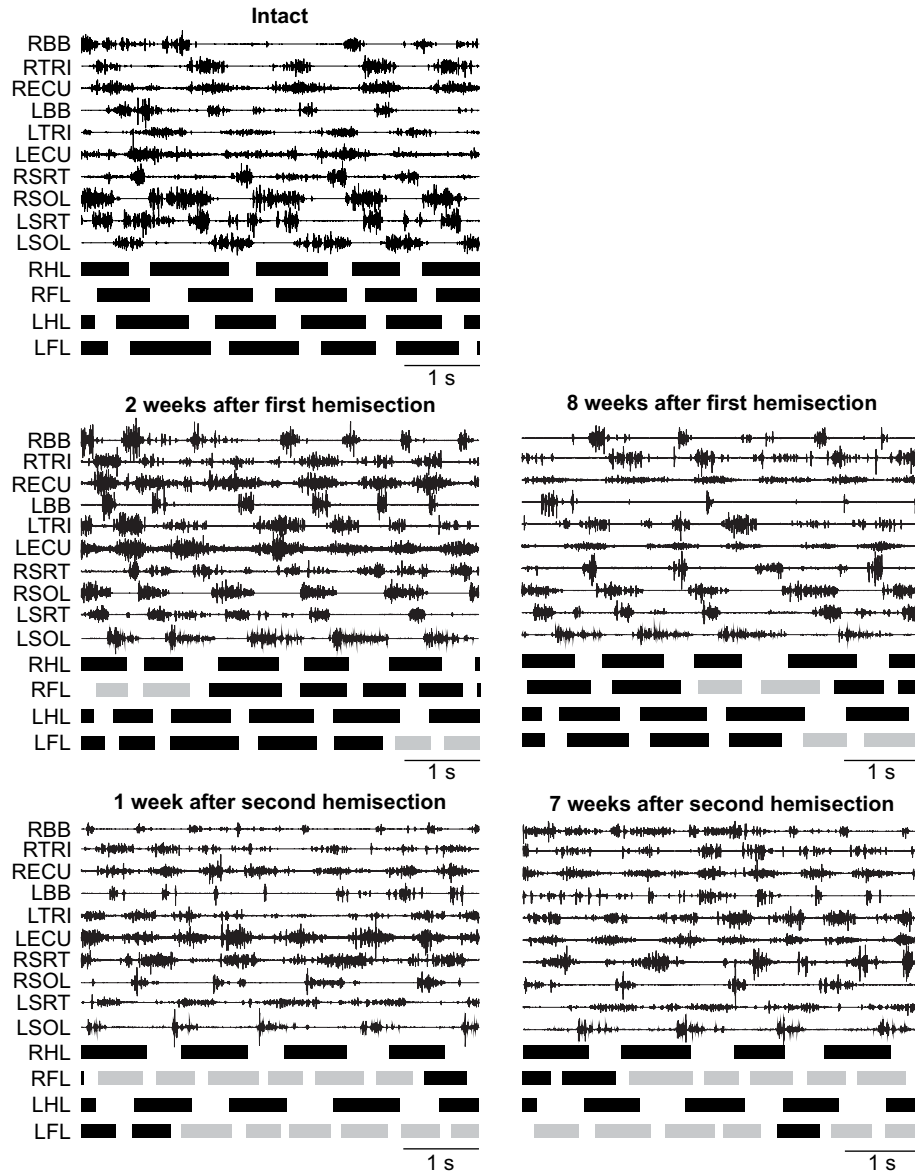


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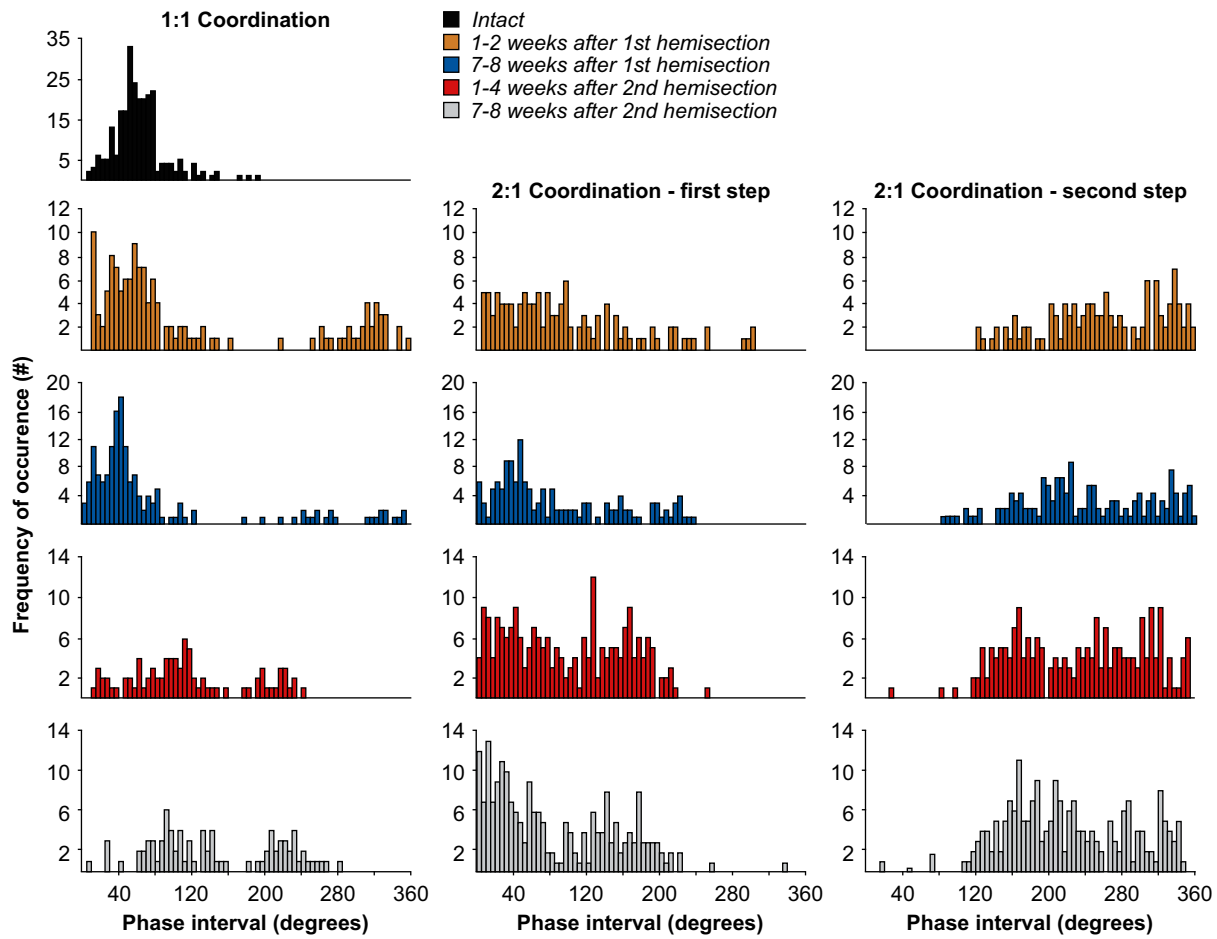


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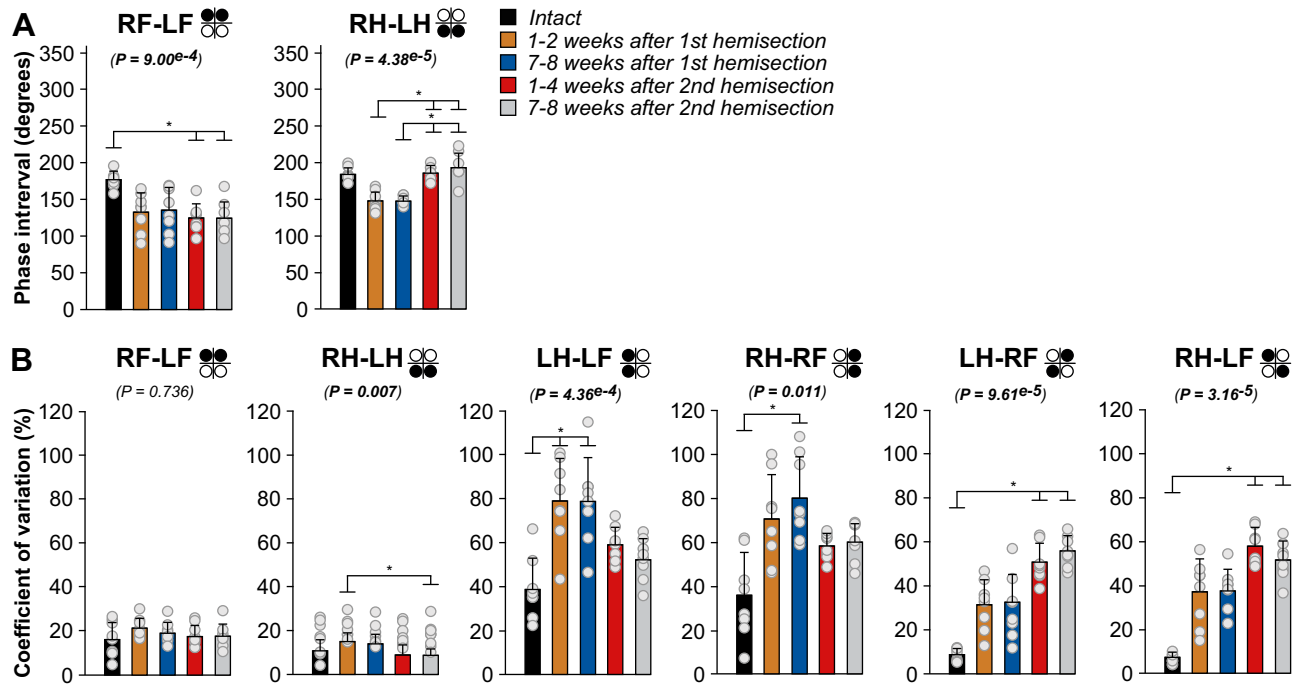


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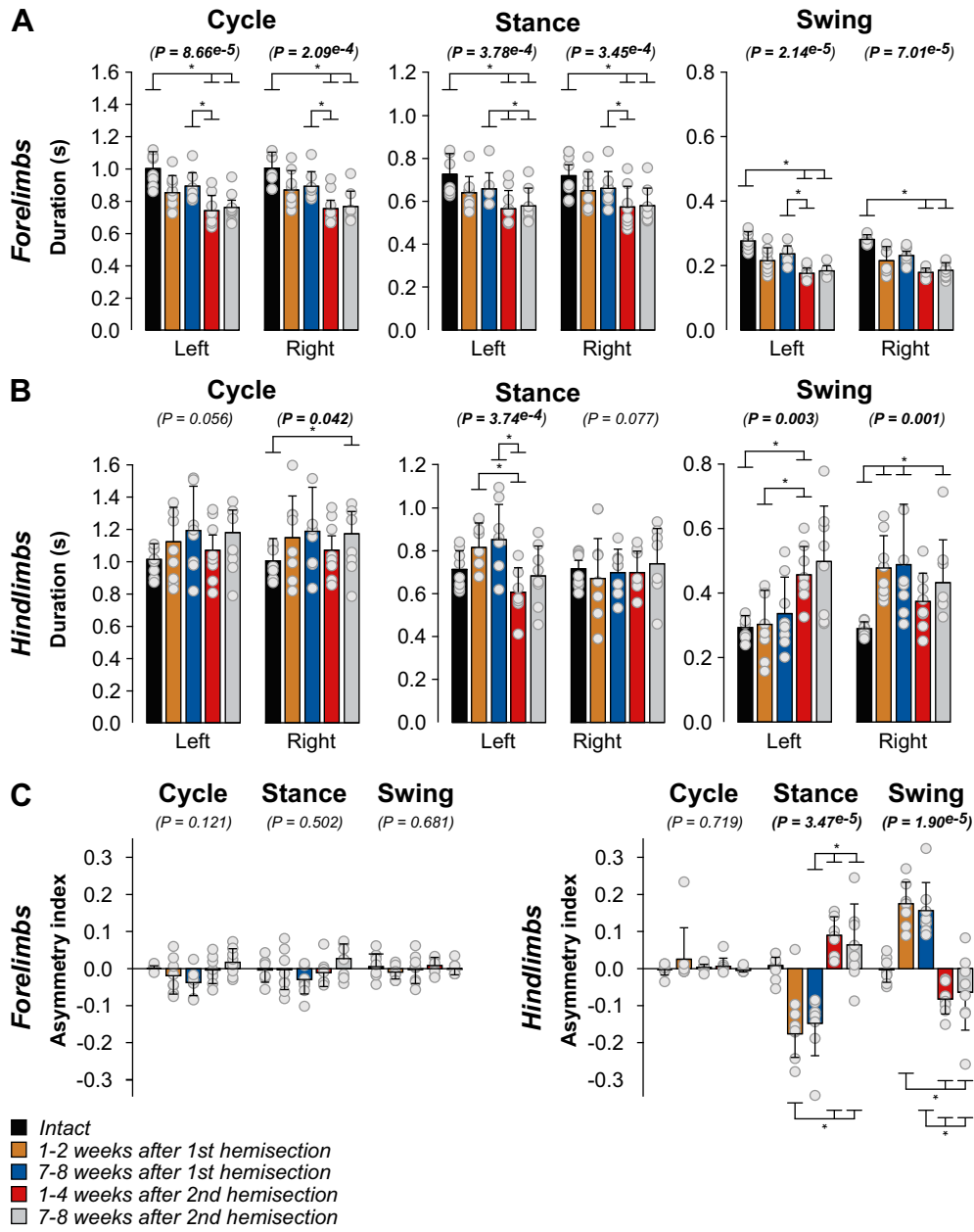


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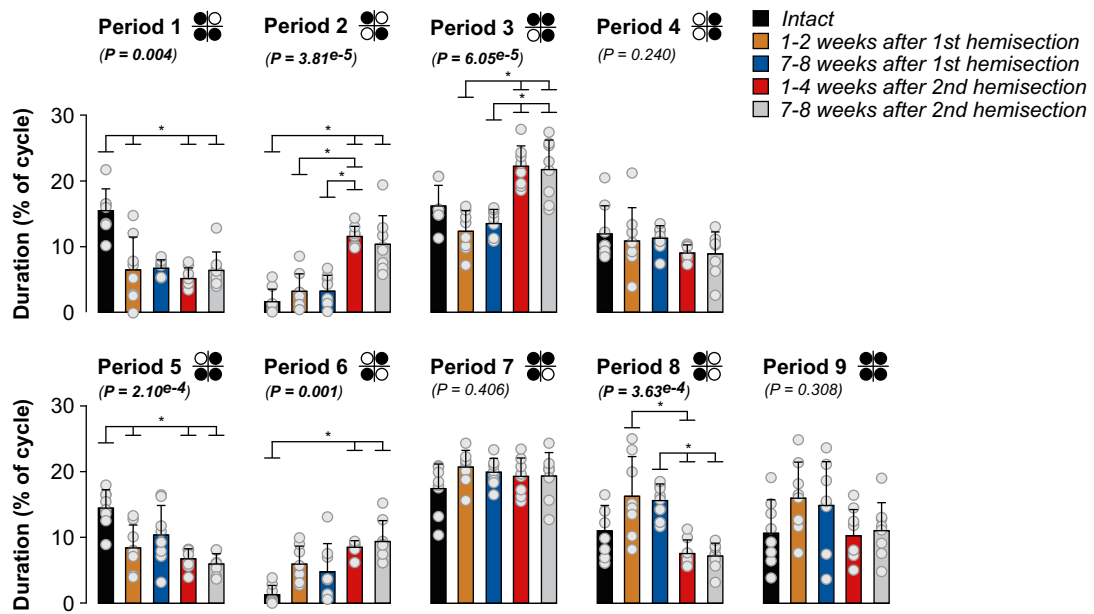


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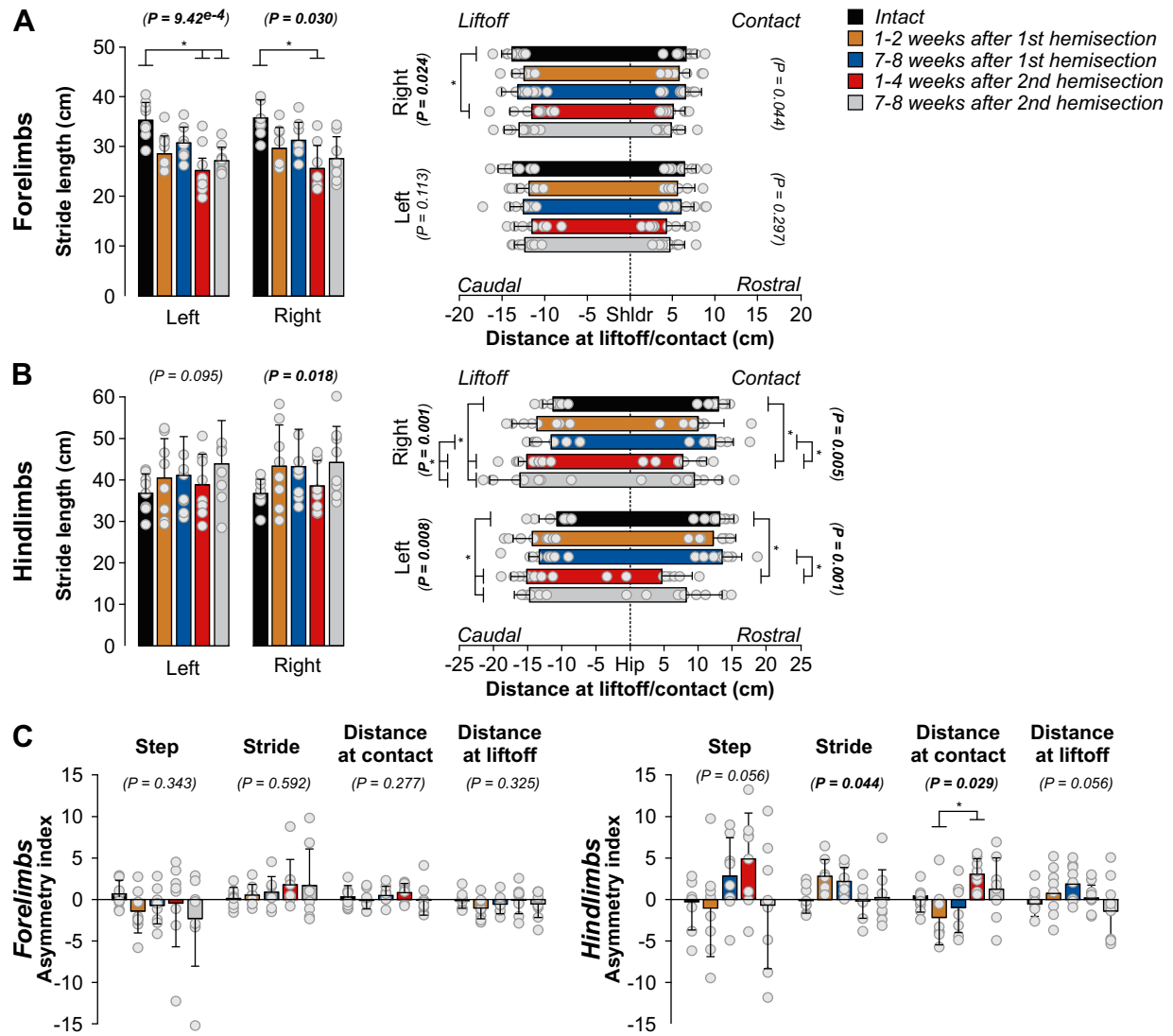


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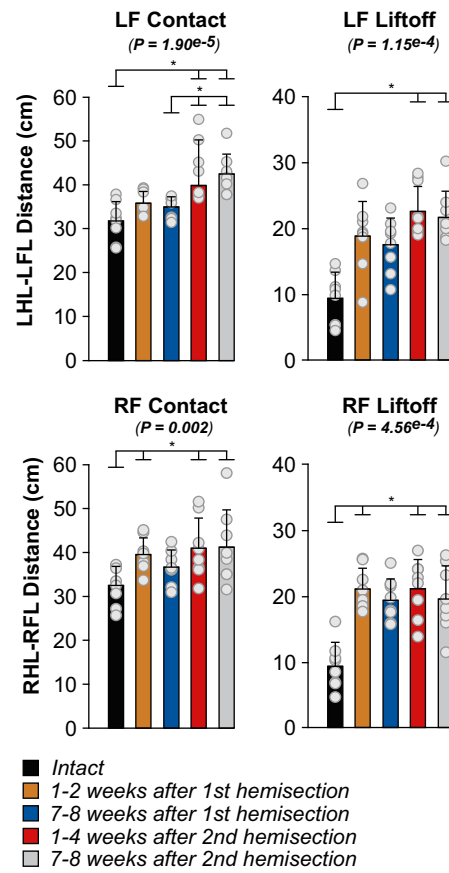


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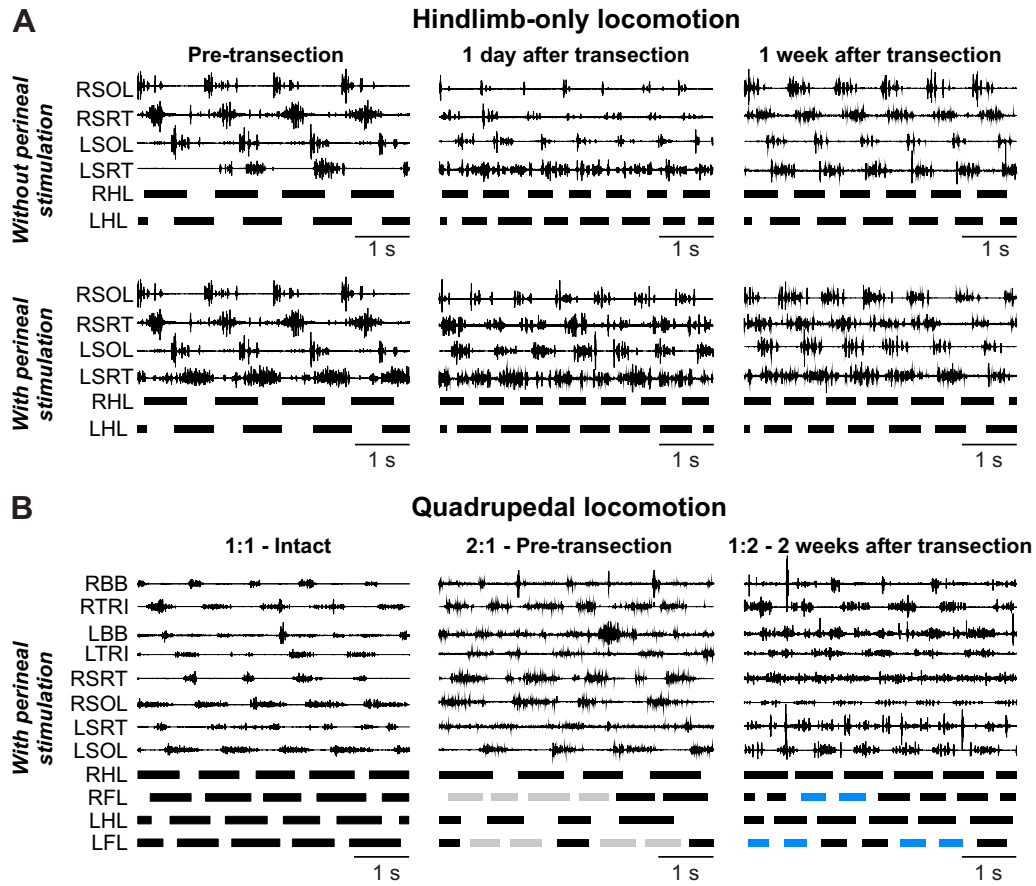


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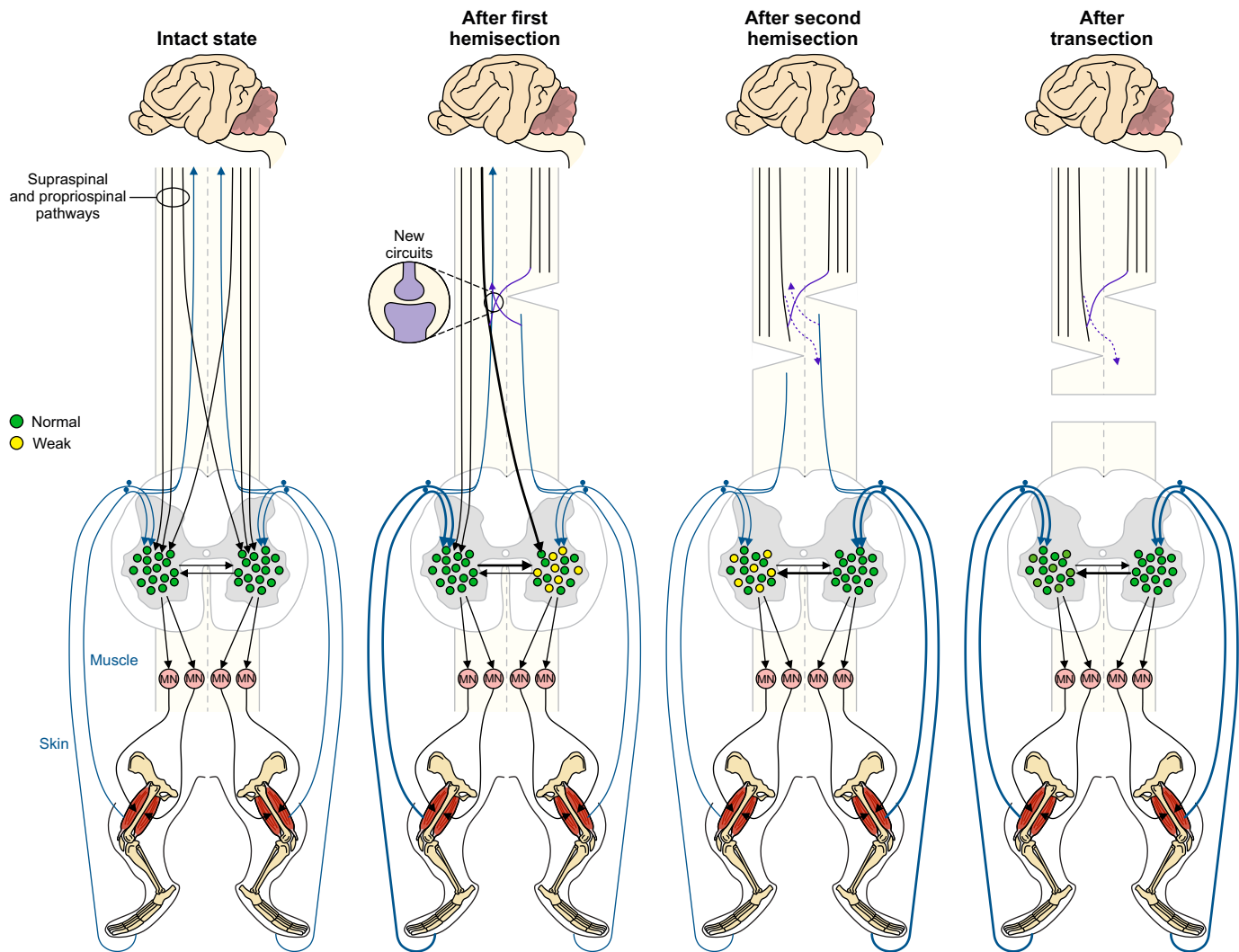


Figure 10