

1 **High-density spinal cord stimulation selectively activates lower urinary tract afferents**

2 Authors: Maria K Jantz<sup>1,2,3</sup>, Chaitanya Gopinath<sup>1,4</sup>, Ritesh Kumar<sup>1,2,3</sup>, Celine Chin<sup>5</sup>, Liane Wong<sup>5</sup>, John I  
3 Ogren<sup>5</sup>, Lee E Fisher<sup>1,2,3,4</sup>, Bryan L McLaughlin<sup>5</sup>, Robert A Gaunt<sup>1,2,3,4</sup> \*

4

5 <sup>1</sup>Rehab Neural Engineering Labs, University of Pittsburgh, PA

6 <sup>2</sup>Department of Bioengineering, University of Pittsburgh, PA

7 <sup>3</sup>Center for the Neural Basis of Cognition, Pittsburgh, PA

8 <sup>4</sup>Department of Physical Medicine and Rehabilitation, University of Pittsburgh, PA

9 <sup>5</sup>Micro-Leads Inc., Somerville, MA

10

11 \*Corresponding author

12 **Abstract**

13 Epidural spinal cord stimulation (SCS) has recently been reported as a potential intervention to improve  
14 limb and autonomic functions, with lumbar stimulation improving locomotion and thoracic stimulation  
15 regulating blood pressure. We asked whether sacral SCS could be used to target the lower urinary tract.  
16 Here we show that high-density epidural SCS over the sacral spinal cord and cauda equina of anesthetized  
17 cats evokes responses in nerves innervating the bladder and urethra and that these nerves can be activated  
18 selectively. Sacral epidural SCS always recruited the pelvic and pudendal nerves and selectively recruited  
19 these nerves in all but one animal. Individual branches of the pudendal nerve were always recruited as well.  
20 Electrodes that selectively recruited specific peripheral nerves were spatially clustered on the arrays,  
21 suggesting anatomically organized sensory pathways. This selective recruitment demonstrates a mechanism  
22 to directly modulate bladder and urethral function through known reflex pathways, which could be used to  
23 restore bladder and urethral function after injury or disease.

24

## 25 Introduction

26 Lower urinary tract (LUT) dysfunction occurs in 20-40% of the global population<sup>1</sup> and has an economic  
27 impact measured in billions of dollars in medical costs every year<sup>2</sup>. One common clinical problem is  
28 overactive bladder; people experience excessive bladder contractions that increase the frequency with  
29 which they feel the urge to void<sup>3</sup>. Overactive bladder reduces sleep quality and participation in daily  
30 activities, and is associated with increased incidence of urinary tract infections<sup>3</sup>. Furthermore, losing  
31 voluntary bladder control is one of the least visible but most limiting consequences of spinal cord injury  
32 (SCI), making improvements in bladder control one of the highest priorities for people with SCI<sup>4</sup>.  
33 Unfortunately, current treatment methods for people living with neurogenic bladder dysfunction,  
34 particularly catheters, only address symptoms and routinely cause urinary tract infections requiring  
35 hospitalization<sup>5,6</sup>.

36 Electrical stimulation of the nervous system offers the potential to address the underlying causes of  
37 neurogenic bladder dysfunction and recent studies of epidural spinal cord stimulation (SCS) accompanied  
38 by locomotor training have shown improvements in bladder function<sup>7-9</sup>. In fact, in humans, locomotor  
39 training alone improved bladder control<sup>10</sup>, while SCS alone in rats with SCI modulated urethral sphincter  
40 activity<sup>11</sup>. Improvements in LUT control may therefore be driven either indirectly or through direct  
41 recruitment of afferents innervating the bladder. In the case of limb motion, cervical and lumbar SCS can  
42 recruit muscles of the upper and lower limbs<sup>12,13</sup> by activating reflexes<sup>14,15</sup>, demonstrating that focal  
43 stimulation of the afferent system can control motor behaviors<sup>16,17</sup>.

44 Electrical stimulation of the pelvic and pudendal nerve can produce bladder contractions through a variety  
45 of reflex mechanisms<sup>18-22</sup>; stimulating afferents in the pudendal nerve can evoke reflexive micturition<sup>21,23,24</sup>  
46 or suppress ongoing bladder contractions, while afferent activity in the pelvic nerve can modulate this reflex  
47 response<sup>25</sup>. These complex reflexes arise in part due to the multiple peripheral targets of the pudendal nerve.  
48 The pudendal nerve divides distally into the sensory, deep perineal, and caudal rectal branches<sup>22</sup>. The caudal  
49 rectal branch innervates the external anal sphincter and pelvic floor<sup>26</sup>, while the sensory and deep perineal  
50 branches innervate the genitalia and urethra. Stimulation of these branches can either reflexively inhibit or  
51 evoke micturition<sup>18,22</sup>. However, accessing and instrumenting these nerves could be challenging in humans  
52 and will require new surgical procedures, complicating translation of a peripheral nerve-based device<sup>27-30</sup>.

53 In this study we sought to determine whether epidural SCS can selectively activate the peripheral nerve  
54 pathways that control the lower urinary tract. We tested this idea using custom high-density electrode arrays  
55 to maximize the opportunity to activate focal sensory inputs while minimizing recruitment of unwanted  
56 reflexes. Lower-limb activation frequently accompanies stimulation at the lumbosacral cord<sup>31</sup>, potentially  
57 arising from the design of existing SCS arrays that often cover an entire segment of the spinal cord with  
58 just a few electrodes<sup>32</sup>. If selective activation were possible, this would establish high-density SCS as a  
59 method to directly modulate LUT function by activating sensory afferents in the pudendal and pelvic  
60 nerves.

## 61 Results

62 To test whether high-density SCS could recruit peripheral nerves that innervate the lower urinary tract, we  
63 stimulated through each contact on the electrode arrays while they were positioned over the sacral spinal  
64 cord and cauda equina (Figure 1). We measured antidromic recruitment of afferents in the pelvic, pudendal  
65 and sciatic nerves, and used the relative recruitment of these nerves to determine selectivity in six  
66 anesthetized cats. These pathways are co-activated in normal function<sup>18,33</sup> so we also measured the co-  
67 recruitment of all these nerves at increasing stimulation amplitudes.

### 68 *High-density SCS selectively recruits pelvic and pudendal afferents*

69 High-density epidural SCS selectively recruited both the pelvic and the pudendal nerves at the sacral cord  
70 and cauda equina in all but one animal. Surprisingly, we found that individual electrodes within the array

71 could selectively recruit different nerves even though the electrodes were often less than 1 mm apart. As a  
72 typical example (animal 5, Figure 2), an individual electrode selectively recruited the pelvic nerve at 390  
73  $\mu\text{A}$  and the pudendal nerve was not recruited until the amplitude was increased to 460  $\mu\text{A}$  (Figure 2a,b). A  
74 nearby electrode recruited the pudendal nerve selectively at 280  $\mu\text{A}$  (Figure 2c).

75 We characterized nerve recruitment in three ways: selective recruitment at the threshold amplitude, total  
76 recruitment at the threshold amplitude, and recruitment at the maximum stimulation amplitude.

77 There was no difference in the threshold amplitudes required to selectively recruit the pelvic and pudendal  
78 nerves ( $n=95$  selective trials,  $p=0.31$ , Wilcoxon test), with pelvic nerve thresholds ranging between 150-  
79 600  $\mu\text{A}$  and pudendal nerve thresholds ranging between 150-690  $\mu\text{A}$  (Figure 3a, filled circles). Similarly,  
80 there was no difference in the threshold amplitude when the pelvic and pudendal nerves were not recruited  
81 selectively ( $n=221$  non-selective trials,  $p=0.28$ , Wilcoxon test) and ranged between 100-800  $\mu\text{A}$  (Figure  
82 3a,b). While there was no difference in the recruitment thresholds between the pelvic and pudendal nerves,  
83 when all nerves were considered, there was a significant difference in the threshold amplitudes across  
84 subjects ( $n=6$  subjects,  $p<0.001$ , Kruskal-Wallis test, Figure 3c) and spinal locations ( $p<0.001$ , Kruskal-  
85 Wallis test, Figure 3d). With the arrays placed at the level of the L6 and S1 vertebrae, the threshold  
86 amplitudes were lower than with the arrays placed at the L7 vertebra ( $p<0.001$ , Dunn's test, Figure 3d) and  
87 placing the arrays at the L6 vertebra resulted in lower threshold amplitudes than when they were placed  
88 under the S1 vertebra ( $p=0.001$ , Dunn's test).

89 The pelvic nerve was recruited selectively at 11 of the 14 tested array locations across the five animals in  
90 which selective recruitment occurred. Selective recruitment of the pelvic nerve occurred most often when  
91 the array was at the level of the S1 dorsal process (five animals) and occurred on 8.3% of the electrodes  
92 ( $n=368$  trials) at this level (Figure 4, dark green bars, Table 1). The pelvic nerve was also recruited  
93 selectively in three animals at the L6 and L7 laminar levels (Table 1).

94 The pudendal nerve was recruited selectively at 11 of the 14 tested array locations across the five animals  
95 in which selective recruitment occurred. Selective recruitment occurred most often with the array at the L6  
96 vertebra (five animals) and occurred on 13.5% of the electrodes at this level (Figure 4, dark purple bars).  
97 In 3 animals the pudendal nerve was also recruited selectively at the lower two levels (Table 1).

98 On some SCS electrodes the threshold stimulation amplitude evoked activity in multiple peripheral nerves  
99 simultaneously. Therefore, we also measured the combined selective and non-selective recruitment at the  
100 threshold amplitude. Lastly, we measured nerve recruitment through SCS electrodes at high amplitudes  
101 (well above the threshold amplitude) to characterize the maximum recruitment potential of an individual  
102 electrode. The pelvic nerve was recruited at threshold on 27.5% of the electrodes across all placements  
103 (Figure 4, light green bars, Table 2) and at the maximum stimulation amplitude on 82.3% of the electrodes  
104 (see Table 3 for additional detail). The pudendal nerve was recruited at threshold on 41.3% of the electrodes  
105 across all placements (Figure 4, light purple bars, Table 2) and at the maximum amplitude on 84.2% of  
106 electrodes (see Table 3 for additional detail). On 14.0% of the electrodes, or about 3-4 electrodes on a 24-  
107 channel array, stimulation at maximal intensities evoked no detectable response on either the pelvic or  
108 pudendal nerves.

### 109 *Recruitment of pudendal nerve branches*

110 Activating different branches of the pudendal nerve can have different effects on bladder function<sup>22,24</sup>, and  
111 we were therefore interested in determining the recruitment properties of these individual branches  
112 (sensory, deep perineal, and caudal rectal). SCS evoked responses in every branch of the pudendal nerve in  
113 every cat at the maximal stimulation amplitude (Table 3). There was no difference in the number of  
114 electrodes that could recruit these nerves at different spinal levels at these high intensities ( $p=0.989$ ,  
115 Kruskal-Wallis test).

116 Pudendal nerve branches were recruited at the threshold amplitude most often at the S1 location (Figure 4,  
117 blue bars). At this location, all three branches were recruited at threshold in every animal (Table 2) and the  
118 caudal rectal and deep perineal nerves were significantly more likely to be recruited at threshold compared  
119 to other locations ( $p < 0.005$ , Kruskal-Wallis test), although this difference was not significant for the  
120 sensory branch ( $p = 0.069$ , Kruskal-Wallis test).

121 Selective recruitment of the pudendal nerve branches was rare in all animals at all spinal locations (Table  
122 1). The sensory branch was recruited selectively at just five of the 17 total tested locations. The other two  
123 branches were each selectively recruited at only two of the 17 locations, with the deep perineal branch  
124 recruited selectively on just 13 electrode contacts across all experiments and the caudal rectal branch  
125 recruited selectively on just three contacts.

126 Lastly, the stimulation amplitude required to recruit the deep perineal and caudal rectal nerves was  
127 significantly higher than all other nerves at the L6 and L7 laminar levels (n=97 L6 trials and 93 L7 trials,  
128  $p < 0.001$ , Kruskal-Wallis test, Figure 3a). This was not true at the S1 lamina level (n=97 trials,  $p = 0.360$ ,  
129 Kruskal-Wallis test, Figure 3a).

### 130 *Sciatic nerve recruitment*

131 A common side effect of electrically stimulating the sacral cord or nerves is lower limb movement resulting  
132 from sciatic nerve activation<sup>7,11,34,35</sup>. Therefore, we monitored sciatic nerve activity during these  
133 experiments and found that similar to the results for the pelvic and pudendal nerves, SCS activated the  
134 sciatic nerve both selectively and non-selectively at all levels. The sciatic nerve had lower thresholds than  
135 all nerves except for the pudendal nerve at the L6 location (n=113 trials,  $p < 0.001$ , Kruskal-Wallis test) and  
136 lower thresholds than all nerves at the L7 location (n=101 trials,  $p < 0.001$ , Kruskal-Wallis test). However,  
137 sciatic threshold amplitudes were no different than the other nerves at the S1 lamina location (n=106 trials,  
138  $p = 0.360$ , Kruskal-Wallis test).

139 These lower thresholds compared to LUT nerves are likely responsible for the fact that at the L6 and L7  
140 laminar locations, the sciatic nerve was recruited selectively more often than any LUT nerve (Figure 4, dark  
141 red bars). However, at the S1 location, there was no difference in the number of electrodes that selectively  
142 recruited LUT and sciatic nerves ( $p = 0.185$ , Kruskal-Wallis test).

### 143 *Nerve selectivity changes with stimulation location*

144 In most animals the pelvic, pudendal, and sciatic nerves were selectively recruited by multiple electrodes.  
145 We investigated the extent to which these patterns of recruitment tended to be organized within the array.  
146 Figure 5a shows a representative example of the nerves selectively recruited by individual electrodes across  
147 the three array levels in one animal. Many electrodes recruited nerves selectively (Figure 5a, colored  
148 rectangles), while other electrodes only recruited nerves non-selectively (Figure 5a, black rectangles). No  
149 obvious organization was seen when we considered only purely selective electrodes. However, when we  
150 examined both selective and non-selective responses at threshold, we observed stimulation ‘hot spots’  
151 within the arrays (Figure 5b). To quantify similarities in spatial recruitment we compared the nerves  
152 recruited on neighboring electrodes to the nerves recruited by each individual electrode. When we  
153 stimulated through an electrode that activated a particular nerve at threshold, 62.5% (IQR 38.2-80.0%) of  
154 the neighboring electrodes recruited that same nerve at threshold (n=320 trials). Conversely, if an electrode  
155 did not recruit a nerve at threshold, the neighboring electrodes were also unlikely to recruit that nerve at  
156 threshold, with a median recruitment of 0.0% (IQR 0.0-20.0%) (n=318 trials, Figure 5c, gray bars). Even  
157 though individual electrodes were very close to each other (0.23-0.78 mm), 37.5% (IQR 0.0%-62.5%) of  
158 adjacent electrodes recruited at least one different nerve. Every nerve was recruited more frequently at  
159 threshold when it was also recruited at threshold on an adjacent electrode (Figure 5c, colored bars).

160 This relationship occurred not only at threshold, but was also true at the maximum stimulation amplitude.  
161 At the maximum amplitude, if an electrode recruited a nerve, 84% of surrounding electrodes also recruited

162 that nerve (n=320 trials). However, if an electrode did not recruit a nerve, only 51% of surrounding  
163 electrodes did (n=149 trials).

#### 164 *Nerve coactivation*

165 Because we observed numerous electrode contacts with non-selective nerve recruitment, we wanted to  
166 quantify the extent to which this recruitment was limited to LUT nerves as compared to co-activation with  
167 the off-target sciatic nerve. This co-activation of different groups of nerves varied by level.

168 At the L6 and L7 levels, the sciatic nerve was coactivated with the LUT nerves on most occasions (Fig.  
169 6a,b). Given the overall lower recruitment of the sciatic nerve at the S1 lamina level, coactivation was much  
170 less common at this level (Fig. 6c). Conversely, the pelvic and pudendal nerves were coactivated more  
171 frequently at the S1 level (Fig. 6c) than at the two more rostral spinal locations (Figure 6a,b). When the  
172 pudendal nerve was recruited, the pelvic nerve was also active 30.3%, 33.3% and 69.3% of the time at the  
173 L6, L7, and S1 locations respectively.

#### 174 *Dynamic range of recruited nerves*

175 While the primary aim of this experiment was to identify whether peripheral afferents could be recruited  
176 selectively by SCS, we also wanted to characterize the dynamic range of stimulation on each electrode. The  
177 dynamic range is the stimulus amplitude range between the threshold amplitude and the stimulation  
178 amplitude at which additional nerves are recruited. Within this range, stimulation remains selective. The  
179 dynamic range was typically smallest with the array placed at the S1 lamina level ( $p < 0.001$ , Kruskal-Wallis  
180 test, Figure 7) and had a median of 20  $\mu\text{A}$  across all electrodes and animals. With the array placed at the L6  
181 and L7 laminar levels, the median dynamic range was 50  $\mu\text{A}$ .

182 The dynamic range also varied between different nerve groups. The amplitude difference between LUT  
183 nerve recruitment and coactivation with other LUT nerves was 20  $\mu\text{A}$  (IQR 10-30  $\mu\text{A}$ ) (n=149 trials). For  
184 LUT nerves to become coactivated with the sciatic nerve, the dynamic range was slightly higher at 30  $\mu\text{A}$   
185 (IQR 10-50  $\mu\text{A}$ ) (n=47 trials). Finally, when the sciatic nerve was recruited selectively, an amplitude  
186 increase of 80  $\mu\text{A}$  (IQR 40-140  $\mu\text{A}$ ) was required to recruit LUT nerves (n=115 trials).

## 187 **Discussion**

188 We found that epidural SCS over the sacral cord can recruit afferent axons arising from the pelvic and  
189 pudendal nerves, providing a mechanism to directly modulate bladder function. While completely selective  
190 recruitment of LUT nerves was not common (Fig. 4), selective recruitment of the pelvic, pudendal, and  
191 sciatic nerves was possible at all three spinal levels tested and in five of the six animals.

192 Epidural SCS has been explored in combination with locomotor training to improve lower urinary tract  
193 control in humans<sup>7</sup> and rats<sup>8</sup>. However, it was unclear from these studies whether the benefits of stimulation  
194 on bladder function arose directly from SCS itself, or whether the benefits were primarily driven by indirect  
195 effects such as improved mobility. Our results demonstrate a plausible physiological mechanism for SCS  
196 to directly recruit LUT reflexes and modulate bladder function. In these experiments, both pelvic and  
197 pudendal nerves were activated by SCS and stimulating pelvic nerve<sup>36,37</sup> and pudendal nerve<sup>18,21-23</sup> afferents  
198 can facilitate or inhibit micturition. While it might seem more obvious to directly target the pelvic and  
199 pudendal nerves for stimulation, surgical access to these nerves can be challenging in humans, and the  
200 pelvic nerve is particularly inaccessible<sup>38,39</sup>. Here, we demonstrate that it is possible to access LUT afferent  
201 pathways using sacral SCS, although the degree to which it is important to selectively activate specific  
202 branches remains unclear.

203 We have further demonstrated in this study that high-density spinal cord arrays are able to produce  
204 completely different recruitment patterns within the same spinal level simply by changing the active  
205 electrode within the array. This result suggests that high-density electrode arrays could be particularly

206 beneficial to optimize SCS for improving bladder function—or any other function of interest at other spinal  
207 levels<sup>40–45</sup>—and that existing commercial stimulation leads may be inadequate.

208 We placed our stimulation electrodes over the sacral spinal cord rather than more rostral levels of the cord,  
209 where many clinical implants are placed<sup>46–49</sup> as the sacral cord contains the motoneuron pools of the bladder  
210 and urethral sphincter<sup>50</sup> and gives rise to the entirety of the pelvic and pudendal nerves<sup>51–53</sup>. While there  
211 were some differences between stimulation locations in terms of threshold amplitudes (Figure 3d),  
212 selectivity (Figure 4) and dynamic range (Figure 7), these differences were subtle. In fact, within any given  
213 animal, we were able to recruit all instrumented nerves at all levels.

214 We evoked the most activity in pelvic afferents when the array was at more caudal locations while pudendal  
215 nerve afferents were recruited at more rostral locations (Figure 4). This is consistent with previous  
216 anatomical observations of the roots that contribute to each of these nerves. The pudendal nerve is typically  
217 composed of fibers from the S1 and S2 roots, while the pelvic nerve is typically composed of fibers from  
218 the S2 and S3 roots<sup>54,55</sup>. Afferents of the pelvic nerve may however be located more rostrally, in the S1 and  
219 S2 roots, in some animals<sup>56</sup>. Furthermore, motoneuron pools for muscles innervated by the pudendal nerve  
220 tend to be located in the S1 and S2 cord in Onuf's nucleus, while motoneuron pools for muscles innervated  
221 by the pelvic nerve are typically focused in the S2 and S3 cord<sup>50,55</sup>.

### 222 *Lower limb activation*

223 If the sciatic nerve were always activated during stimulus trains intended to recruit LUT nerves, the  
224 associated lower-limb movement could be very disruptive. In fact, activation of the lower limb is a common  
225 problem with the commercially available InterStim sacral nerve stimulators<sup>57</sup> and the Finetech anterior root  
226 stimulation system<sup>58</sup>. Although motor activation of the lower-limb does not prevent bladder prostheses from  
227 being effective, it is typically an undesirable off-target effect<sup>7,11,34,35</sup>. On the other hand, recruiting sensory  
228 afferents of the lower limb, particularly the tibial nerve, has been shown to improve continence<sup>59</sup>, making  
229 this a potentially useful target in some contexts. We found that in the spinally intact cat, there was  
230 substantially less activation of the sciatic nerve when the electrodes were over the cauda equina compared  
231 to the sacral cord, which is consistent with the path of the lower lumbar and sacral roots within the spinal  
232 canal at these locations.

### 233 *LUT co-activation*

234 While this study focused on selective nerve activation, axons in many different LUT nerve branches are  
235 active simultaneously in behavior. For instance, the anal sphincter, innervated by the caudal rectal nerve,  
236 and the external urethral sphincter, innervated by the deep perineal nerve, are frequently coupled<sup>33</sup>. Further,  
237 in some cases, co-stimulation of multiple pudendal branches improves voiding efficiency<sup>24</sup>. It is therefore  
238 likely that selectively activating these branches may not be necessary to effectively control bladder function.  
239 This is encouraging because we found that the dynamic range for selective stimulation was typically less  
240 than 100  $\mu$ A.

241 The sensory branch typically had lower threshold amplitudes than the deep perineal and caudal rectal  
242 branches (Figure 4b). Because SCS primarily recruits afferents, this difference in threshold could be due to  
243 the high density of afferent fibers in the sensory branch compared to both the deep perineal and caudal  
244 rectal branches, which have substantial motor functions<sup>54</sup>.

### 245 *Limitations*

246 In this study we focused on selective recruitment of individual nerves. While we saw that it was possible  
247 to selectively activate most nerves in most animals, the actual number of electrode contacts that selectively  
248 recruited LUT nerves was low. However, this selectivity may not be necessary, as many functional  
249 behaviors require coactivation of multiple pathways. If improved selectivity were directly related to

250 functional control of the LUT, it would be useful to maximize the number of electrode contacts that  
251 selectively recruited LUT afferents.

252 In this study we used monopolar stimulation exclusively, which allowed us to systematically test all the  
253 electrodes in the available time, but may have been suboptimal to recruit afferent populations selectively.  
254 To improve selectively, multipolar stimulation can be used to localize or focus current between several  
255 electrodes<sup>60</sup>. In a recent study in humans, multipolar stimulation was often required to evoke meaningful  
256 sensory percepts in amputees, while monopolar stimulation was generally less effective<sup>61</sup>. Similarly, some  
257 commercially available SCS systems leverage multipolar stimulation to increase the focality of the  
258 paresthesias evoked by stimulation<sup>62</sup>. Additional selectivity could potentially be gained by changing  
259 stimulation waveforms<sup>63,64</sup> or applying variable-frequency stimuli<sup>65</sup>.

260 Another limitation of this study is that it was conducted in acute experiments and we did not determine the  
261 stability of these effects during movement. Postural effects are known to be considerable in human spinal  
262 cord stimulation<sup>47</sup>, and these effects could be exacerbated using small electrodes of the type considered  
263 here. Additionally, this study was performed in cats, and the reduced cerebrospinal fluid thickness  
264 compared to humans may impact parameter choice, particularly threshold amplitudes. The smaller spinal  
265 cord size in cats also requires fewer electrodes to cover the spinal cord area. The large number of electrodes  
266 that could be required in a human application might also require new methods of parameter tuning to be  
267 developed, including closed-loop methods where muscle activity or other non-invasively accessible signals  
268 are used to automatically tune parameters. Ultimately, the goal of this work is to manipulate LUT function,  
269 and here we only study peripheral nerve recruitment, particularly recruitment of the sensory fibers. Future  
270 studies will expand this work to include direct measures of LUT function in response to stimulation.

#### 271 *Implications for neuroprosthetic devices*

272 This study demonstrates that it is possible to selectively activate individual peripheral nerves innervating  
273 the LUT with high-density SCS. This understanding could potentially provide a route to improve upon  
274 recent studies where results may vary considerably between individuals<sup>7</sup>, as it illuminates the variability in  
275 recruitment that could occur with subtly changing electrode positions. This study therefore supports the  
276 design and development of new high-density electrodes to achieve selective activation, which may improve  
277 the effects of human SCS trials. Finally, this study highlights the potential use of epidural SCS to target  
278 autonomic systems generally<sup>66</sup> by adding a physiological and scientific basis for stimulating these  
279 pathways.

## 280 **Methods**

### 281 *Surgical procedures*

282 Acute experiments were conducted under isoflurane anesthesia in 6 adult male cats weighing between 4.1  
283 and 6.4 kg. All procedures were approved by the University of Pittsburgh Institutional Animal Care and  
284 Use Committee. All procedures were performed in accordance with the relevant guidelines and regulations.

285 The animals were anesthetized with a ketamine/acepromazine cocktail and anesthesia was maintained using  
286 isoflurane (1-2%). A tracheostomy was performed and the trachea was cannulated and connected to an  
287 artificial respiration system. Throughout the procedure, the animal was artificially ventilated at 12-14  
288 breaths per minute. Blood pressure was monitored with a catheter placed in the carotid artery. Temperature  
289 was maintained with a warm air heating pad and IV fluids were administered continuously. End tidal CO<sub>2</sub>,  
290 SpO<sub>2</sub>, core temperature, heart rate and blood pressure were monitored throughout the procedure and kept  
291 within a normal physiological range. Following experimental data collection, animals were euthanized with  
292 an IV injection of Euthasol.

293 The bladder was exposed through a midline abdominal incision and a dual-lumen catheter (Model CDLC-  
294 6D, Laborie Medical Technologies, Williston, VT) was placed through the bladder dome to measure

295 bladder pressure as well as to infuse and withdraw fluids. The catheter was secured in place with a purse  
296 string suture.

297 To measure antidromic compound action potentials evoked by spinal cord stimulation, we placed bipolar  
298 nerve cuffs (Micro-Leads Inc., Somerville, MA) on the left pelvic and pudendal nerves as well as pudendal  
299 nerve branches (Figure 1a). The pelvic nerve was dissected free near the internal iliac artery and a cuff was  
300 placed prior to the branching of the pelvic plexus. The abdominal incision was then closed in layers and the  
301 animal was placed in the prone position. We made an incision on the left hindquarters between the base of  
302 the tail and the ischial tuberosity and performed blunt dissection to expose the pudendal nerve. We then  
303 placed nerve cuffs on the left pudendal nerve and the sensory, deep perineal, and caudal rectal branches<sup>22</sup>  
304 of the left pudendal nerve. We also placed a five-pole spiral nerve cuff (Ardiem Medical, Indiana, PA) on  
305 the left sciatic nerve to measure off-target effects associated with the lower-limb. A recording reference  
306 electrode, consisting of a stainless steel wire with ~1 cm of insulation removed, was placed subcutaneously  
307 in the left lower back.

308 We performed a laminectomy at the L6, L7, and S1 vertebral levels to expose the sacral spinal cord, then  
309 placed a custom epidural spinal cord array (Micro-Leads Inc., Somerville, MA) with 16 or 24 channels  
310 (Figure 1b,c) on the spinal cord at three different locations over the sacral spinal cord and cauda equina  
311 (Figure 1a). The electrodes on the 16-channel array were each 0.45 mm x 1.35 mm and were spaced 0.69  
312 mm apart laterally and 1.64 mm apart rostrocaudally (Figure 1b, inset shown to scale). The electrodes on  
313 the 24-channel array were each 0.29 mm x 1.0 mm and were spaced 0.23 mm apart laterally and 0.78 apart  
314 rostrocaudally (Figure 1c, inset shown to scale). The centers of the L6, L7 and S1 dorsal spinal processes  
315 were marked using a suture placed in paraspinal muscles prior to the laminectomy. The epidural arrays  
316 were then placed on the epidural surface of the spinal cord and aligned to these suture markers. For the  
317 most rostral location, the arrays were placed such that the most rostral electrode on the array was aligned  
318 with the center of the L6 dorsal process. After experiments were completed at this location the electrode  
319 array was moved so that the most rostral electrode on the array was aligned with the L7 suture marker. For  
320 the final location, the most caudal electrode on the array was aligned with the S1 suture marker. A  
321 stimulation return electrode, consisting of a stainless steel wire with ~1 cm of insulation removed, was  
322 placed outside the spinal column, near the L7 transverse process. Landmarks and dorsal root entry zones  
323 were verified postmortem.

324 Five animals were tested at three spinal levels and one animal tested at two spinal levels (L6 and S1), giving  
325 a total of 17 sets of data.

### 326 *Neural recording and stimulation*

327 Stimulation was delivered with a Grapevine Neural Interface Processor through a Nano 2+ Stim high-  
328 current headstage (Ripple LLC), with stimulation patterns commanded from MATLAB (MathWorks Inc,  
329 Natick, MA). This headstage delivers stimulation current amplitudes of up to 1.5 mA and has a compliance  
330 voltage of  $\pm 8.5$  V. The stimulation amplitude across all trials ranged from 10-1500  $\mu$ A with a resolution of  
331 10  $\mu$ A between steps up to 1280  $\mu$ A, and a resolution of 20  $\mu$ A from 1280-1500  $\mu$ A. Stimulation pulses  
332 were symmetric with 200  $\mu$ s cathodal and anodal phases. Phases were separated by a 66  $\mu$ s interphase  
333 interval. For animals 1-2 and 5-6, the cathodal phase was applied first, followed by the anodal phase. For  
334 animals 3-4, the anodal phase was applied first. Regardless of which phase was applied first, the recruitment  
335 thresholds were no different ( $p=0.28$ , Wilcoxon test).

336 Compound action potentials were sampled at 30 kHz with a Surf S2 headstage (Ripple LLC) through the  
337 Grapevine Neural Interface Processor. The signal was filtered with a high-pass filter with a 0.1 Hz cutoff  
338 followed by a low-pass filter with a 7.5 kHz cutoff, using 3<sup>rd</sup> order Butterworth filters. The signals from  
339 each pole of the bipolar nerve cuffs were then differenced to find the response on a given nerve.



### 340 *Compound action potential detection*

341 Stimulation artifacts were removed from the nerve cuff recordings by linearly interpolating between the  
342 sample immediately before the onset of each stimulus pulse to 0.5 ms after the end of each stimulus pulse.  
343 We then high-pass filtered the signal at 300 Hz using a 2<sup>nd</sup> order Butterworth filter. The signal-to-noise  
344 ratio for detecting antidromic action potentials at the recruitment threshold is substantially less than one  
345 due to the presence of spontaneous activity in the nerves as well as general recording noise. Therefore, we  
346 used stimulus-triggered averaging to detect responses evoked by stimulation. The presence of a compound  
347 action potential on each nerve was determined by comparing responses following stimulation to baseline  
348 recordings in which no stimulation occurred, using a previously-published method<sup>67,68</sup>. To determine the  
349 response detection threshold, we calculated the 99% confidence interval on the root mean squared baseline  
350 amplitude. We then set the detection threshold to 3.2 standard deviations above the upper bound of this  
351 baseline mean, or a minimum of 0.5  $\mu$ V. This threshold was determined empirically to most accurately  
352 detect true responses without false positives. The stimulus-triggered average was calculated 200 times from  
353 a random subsample of 80% of the responses in order to find a distribution of typical responses. In each of  
354 these responses, the root mean squared amplitude of each time window was compared to the root mean  
355 squared of the baseline amplitude, using a 250  $\mu$ s sliding window with a 25  $\mu$ s overlap. If 95% of these  
356 responses were suprathreshold and nerve activity was detected for at least three consecutive windows, the  
357 response was considered significant.

### 358 *Determining recruitment thresholds*

359 A binary search procedure was used to determine the minimum stimulus current necessary to recruit each  
360 nerve according to methods published previously<sup>67,68</sup>. First, we delivered 50 stimulation pulses through  
361 each electrode on the array in a random order using high amplitude pulses at 20 Hz to determine which  
362 electrodes could evoke responses in the peripheral nerves. The stimulation amplitude for this trial was  
363 determined based on the highest amplitude that did not evoke substantial movement in the leg, or when all  
364 electrodes showed neural responses, and typically ranged from 600-1000  $\mu$ A. After the responses to  
365 stimulation at the maximum amplitude were determined, all stimulation electrodes that evoked compound  
366 action potentials in at least one instrumented nerve were tested individually using a binary search procedure  
367 to determine the thresholds for every nerve recruited. We set the stimulation frequency during these trials  
368 by determining the longest-latency neural response on each nerve cuff, adding 5 ms, and taking the inverse  
369 of this time. With this approach, we were able to maximize the stimulation frequency and minimize overall  
370 experiment time. 300 stimulation pulses were delivered to each electrode at each tested amplitude. For each  
371 nerve showing a response, we determined the current threshold to a resolution of 10  $\mu$ A. This procedure  
372 typically took 2-3 hours for each location of the spinal cord array.

373 To determine the selectivity of this high-density SCS electrode, we measured the recruitment thresholds,  
374 selectivity, and dynamic range of stimulation-evoked neural responses. Recruitment thresholds for each  
375 nerve were defined as the lowest amplitude at which a response to SCS was detected. Threshold responses  
376 were considered to be selective when only a single nerve responded at the threshold amplitude and non-  
377 selective when multiple nerves were simultaneously recruited at the threshold amplitude. We determined  
378 whether pudendal nerve branches were activated selectively by excluding the pudendal nerve and  
379 comparing their recruitment thresholds only to other branches, the pelvic nerve, and the sciatic nerve. We  
380 defined the dynamic range of stimulation on an electrode as the difference between the threshold amplitude  
381 for the recruited nerve and the first higher amplitude at which multiple nerves were recruited.

### 382 *Statistics*

383 The recruitment thresholds were not normally distributed ( $p < 0.001$ , Lilliefors test) so data are reported as  
384 the median threshold amplitude with lower and upper quartiles. The Wilcoxon rank-sum test was used to  
385 test for differences between two groups. For comparisons between multiple groups of data, we used a

386 Kruskal-Wallis test with a Dunn's test for post-hoc analysis. The data were analyzed in Matlab 2018a  
387 (Mathworks, Natick, MA).

### 388 **Data Availability**

389 All data collected for this study and used in these analyses are available at [https://doi.org/10.26275/iami-](https://doi.org/10.26275/iami-zirb)  
390 [zirb](https://doi.org/10.26275/iami-zirb).<sup>69</sup> This dataset also contains data from other experiments, so the animals described in this study are  
391 referred to as subjects 54, 60, 64, 63, 68, 69, and 78 in the available dataset, and this study only includes  
392 monopolar stimulation data. These data are provided using the CC BY 4.0 license.

### 393 **Acknowledgements**

394 Research reported in this publication was supported by the Office Of The Director, National Institutes Of  
395 Health of the National Institutes of Health under Award Number OT2OD024908. The content is solely the  
396 responsibility of the authors and does not necessarily represent the official views of the National Institutes  
397 of Health. We thank the veterinary staff at Magee-Womens Research Institute for their help with animal  
398 care and experiments, as well as Massimiliano Novelli for his help with data management and Ameya  
399 Nanivadekar for his advice on data analysis.

### 400 **Author Contributions**

401 RAG and BLM conceived of the overall project. MKJ, CHG, LEF and RAG designed the experiments.  
402 MKJ, CHG, RK and RAG collected the data. LW, JIO, and CC designed and manufactured the custom  
403 electrode arrays. MKJ analyzed the data. MKJ and RAG wrote the manuscript. All authors contributed to  
404 the interpretation of the data and provided critical review and approval of the manuscript.

### 405 **Competing Interests**

406 BLM, LW, CC, and JIO are employees of Micro-Leads Inc. who design and develop implantable electrodes.  
407 The other authors declare no competing interests.

408

409 **Table 1. Selective recruitment of each nerve in all animals by vertebral level.** The percentage of  
410 electrodes at a given array location that selectively recruited each nerve are shown as the median and upper  
411 and lower quartiles. The number of animals where stimulation at each array location selectively recruited  
412 each nerve is also shown.

Nerve	L6 Array Placement		L7 Array Placement		S1 Array Placement	
	% of electrodes	# of animals	% of electrodes	# of animals	% of electrodes	# of animals
Pelvic	2.1 (0.0-16.67)	3/6	6.3 (0.0-16.7)	3/5	8.3 (4.2-18.8)	5/6
Pudendal	13.5 (4.2-25.0)	5/6	16.7 (0.0-39.6)	3/5	2.1 (0.0-16.7)	3/6
Sensory	0.0 (0.0-4.2)	2/6	0.0 (0.0-3.1)	1/5	0.0 (0.0-12.5)	2/6
Deep Perineal	0.0 (0.0-0.0)	0/6	0.0 (0.0-1.0)	1/5	0.0 (0.0-0.0)	1/6
Caudal Rectal	0.0 (0.0-0.0)	1/6	0.0 (0.0-0.0)	0/5	0.0 (0.0-0.0)	1/6
Sciatic	26.0 (8.3-31.3)	5/6	18.8 (7.3-63.5)	5/5	5.2 (0.0-12.5)	4/6

413

414 **Table 2. Total threshold recruitment of each nerve in all animals by vertebral level.** The percentage  
 415 of electrodes at a given array location that recruited each nerve selectively or non-selectively at threshold  
 416 are shown as the median and upper and lower quartiles. The number of animals where stimulation at each  
 417 array location recruited each nerve is also shown.

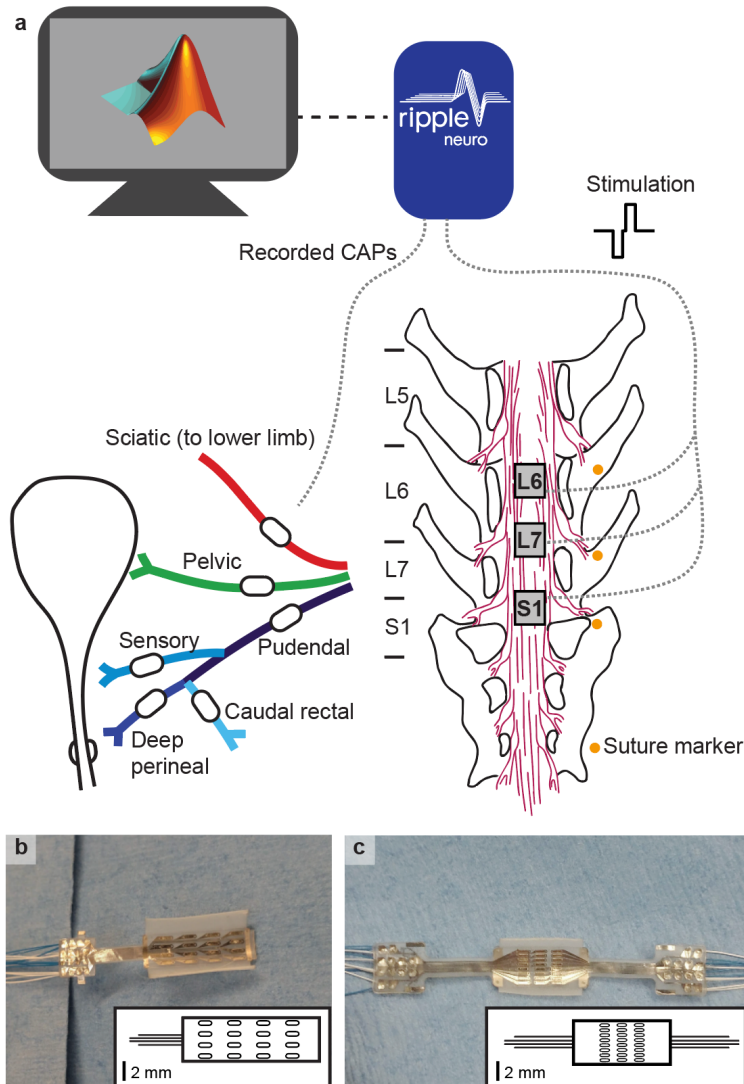
Nerve	L6 Array Placement		L7 Array Placement		S1 Array Placement	
	% of electrodes	# of animals	% of electrodes	# of animals	% of electrodes	# of animals
Pelvic	18.8 (12.5-29.2)	5/6	25.0 (6.3-34.4)	4/5	41.7 (29.2-75.0)	6/6
Pudendal	49.0 (33.3-56.3)	5/6	25.0 (14.1-63.5)	4/5	47.7 (41.7-68.8)	5/6
Sensory	5.2 (0.0-8.3)	4/6	0.0 (0.0-21.9)	2/5	35.4 (20.8-68.8)	6/6
Deep Perineal	0.0 (0.0-4.2)	2/6	0.0 (0.0-1.0)	1/5	28.1 (12.5-62.5)	6/6
Caudal Rectal	2.1 (0.0-4.2)	3/6	0.0 (0.0-1.0)	1/5	18.8 (8.3-31.3)	6/6
Sciatic	54.2 (41.7-75.0)	6/6	37.5 (19.8-63.5)	5/5	12.5 (0.0-12.5)	4/6

418

419 **Table 3. Maximum amplitude recruitment of each nerve in all animals by vertebral level.** The  
 420 percentage of electrodes at a given array location that recruited each nerve at the maximum amplitude are  
 421 shown as the median and upper and lower quartiles. The number of animals where stimulation at each array  
 422 location recruited each nerve is also shown.

Nerve	L6 Array Placement		L7 Array Placement		S1 Array Placement	
	% of electrodes	# of animals	% of electrodes	# of animals	% of electrodes	# of animals
Pelvic	83.3 (75.0-87.5)	6/6	91.7 (80.2-94.3)	5/5	83.3 (62.5-100.0)	6/6
Pudendal	87.5 (62.5-91.7)	6/6	91.7 (84.4-96.9)	5/5	81.3 (66.7-100.0)	6/6
Sensory	87.5 (62.5-91.7)	6/6	91.7 (78.1-96.9)	5/5	83.3 (66.7-100.0)	6/6
Deep Perineal	87.5 (50.0-95.8)	6/6	87.5 (72.9-89.6)	5/5	81.3 (58.3-100.0)	6/6
Caudal Rectal	87.5 (33.3-91.7)	6/6	83.3 (75.5-92.7)	5/5	81.3 (58.3-100.0)	6/6
Sciatic	83.3 (75.0-87.5)	6/6	91.7 (80.2-94.3)	5/5	83.3 (62.5-100.0)	6/6

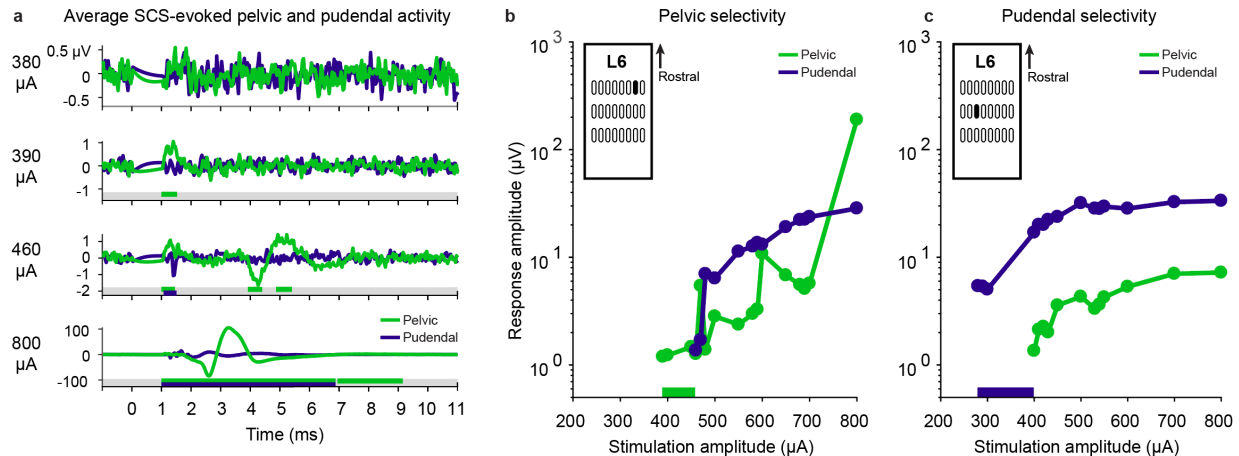
423



424

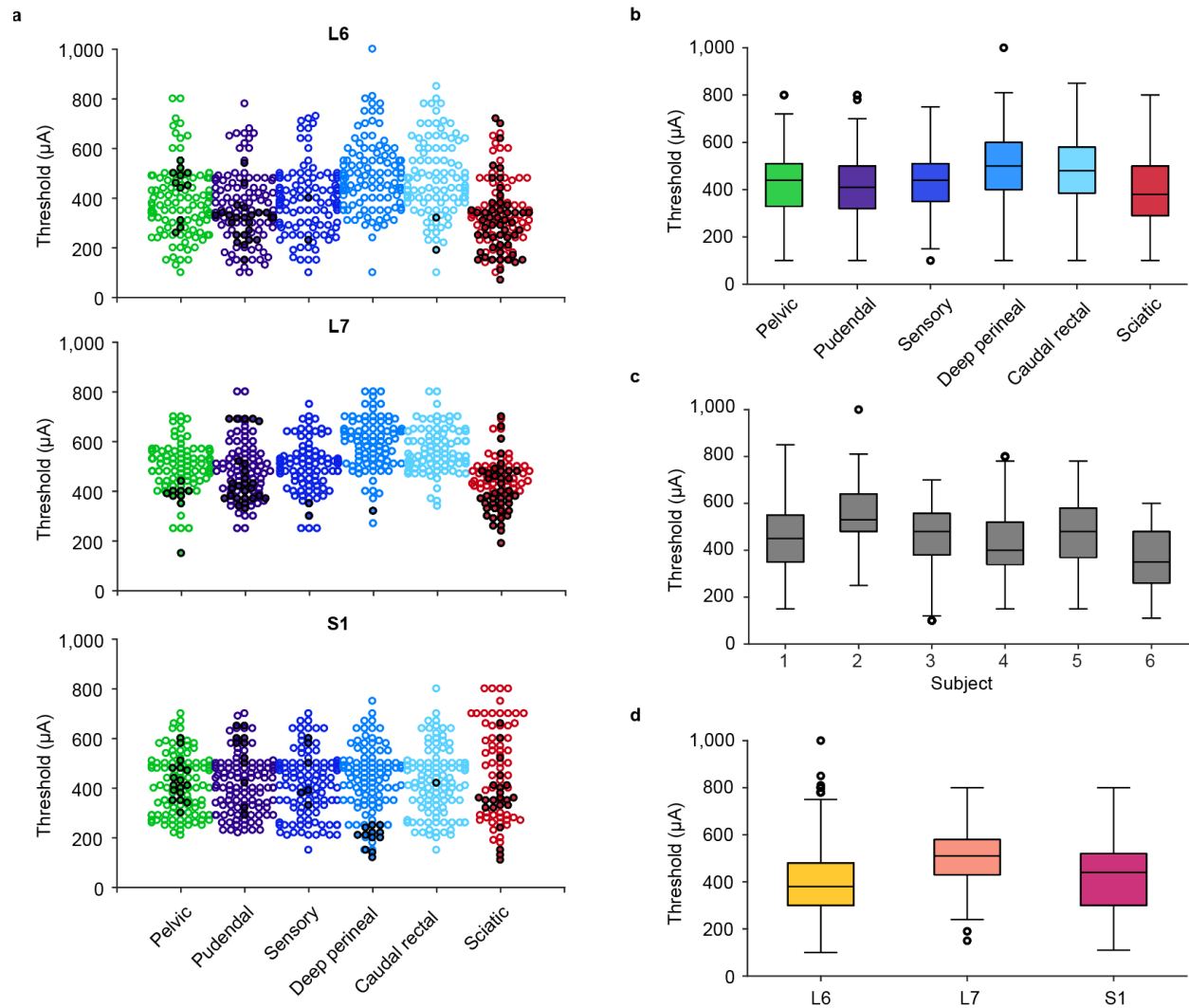
425 **Figure 1. Experimental setup.** a) Nerves cuffs, shown as white ovals on each nerve, were placed on  
426 multiple peripheral nerves and a high-density electrode array was placed at three locations over the sacral  
427 cord and cauda equina. Nerve cuffs on the pelvic nerve (green), pudendal nerve (blue), and pudendal  
428 branches (shades of blue) had an inner diameter between 500  $\mu\text{m}$  and 1000  $\mu\text{m}$ . The sciatic nerve (red) cuff  
429 had an inner diameter of 3 mm. Recording and stimulation were completed through a MATLAB interface  
430 with a Ripple Grapevine system using a closed-loop response detection algorithm. b) In animals 1 and 2, a  
431 16-channel epidural array with four electrode columns spaced laterally across the cord and four electrode  
432 rows spaced rostrocaudally was used. The inset shows the array layout to scale, with the wire bundle  
433 represented in the same orientation as the photo. The electrodes on the 16-channel array were each 0.45  
434 mm x 1.35 mm and were spaced 0.69 mm apart laterally and 1.64 mm apart rostrocaudally. c) In animals  
435 3-6, a 24-channel epidural array with eight columns and three rows was used. The electrodes on the 24-  
436 channel array were each 0.29 mm x 1.0 mm and were spaced 0.23 mm apart laterally and 0.78 apart  
437 rostrocaudally.

438



439

440 **Figure 2. Examples of selective pelvic and pudendal nerve recruitment in animal 5 during stimulation**  
441 **on two different electrodes.** a) Stimulation-triggered averages of the pelvic (green) and pudendal (purple)  
442 nerve compound nerve action potentials at selected stimulation amplitudes. The traces include 1 ms  
443 preceding the stimulus pulse. Windows in which responses were detected are indicated under each trace as  
444 colored bars. At 380  $\mu\text{A}$ , no response was detected in either nerve. At 390  $\mu\text{A}$ , a selective response was  
445 detected in the pelvic nerve. At 460  $\mu\text{A}$  the pudendal nerve was also recruited. Additional responses in the  
446 pelvic nerve at longer latencies also occurred. 800  $\mu\text{A}$  was the maximum stimulation amplitude for this trial  
447 and evoked large compound action potentials in the pelvic nerve. Note the different y-axis scales for each  
448 stimulation amplitude. b) Peak-to-peak compound action potential amplitude of the pelvic and pudendal  
449 nerves for the electrode illustrated in a) that was selective for the pelvic nerve from 390  $\mu\text{A}$  up to 460  $\mu\text{A}$   
450 (selective range highlighted in bar along the x-axis). Only the pudendal and pelvic nerve traces are shown  
451 here, but this electrode did not recruit any other instrumented nerves at threshold. The y-axis is shown on a  
452 log scale. The specific stimulation electrode is highlighted in the inset. c) Peak-to-peak compound action  
453 potential amplitude of the pelvic and pudendal nerves for a nearby electrode (see inset) that selectively  
454 recruited the pudendal nerve from 280  $\mu\text{A}$  up to 400  $\mu\text{A}$  (selective range highlighted in bar along the x-  
455 axis). The y-axis is shown on a log scale.

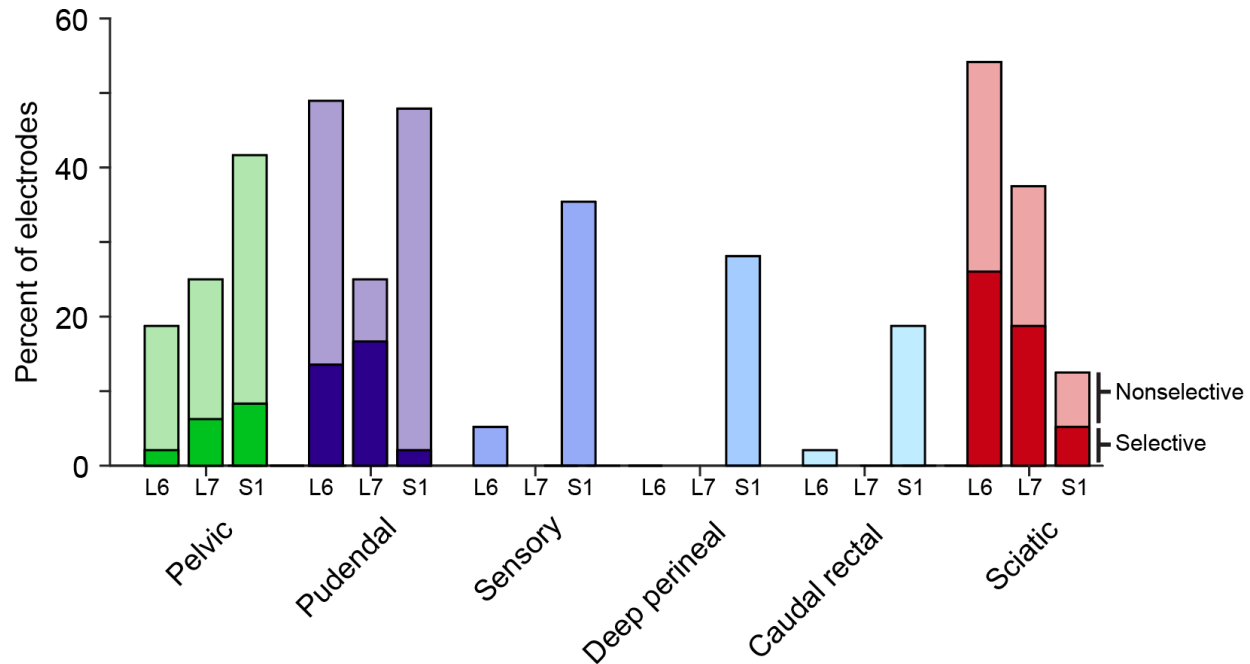


456

457 **Figure 3. Recruitment thresholds for all animals, nerves and locations.** a) Recruitment thresholds for  
458 each nerve at each location across all animals. Trials that recruited nerves non-selectively at the threshold  
459 amplitude are marked with unfilled circles and trials that recruited nerves selectively at the threshold  
460 amplitude are marked with filled circles. b) Recruitment thresholds for each nerve across all locations and  
461 animals. c) Recruitment thresholds for each animal across all nerves and locations. d) Recruitment  
462 thresholds for each location across all nerves and animals.

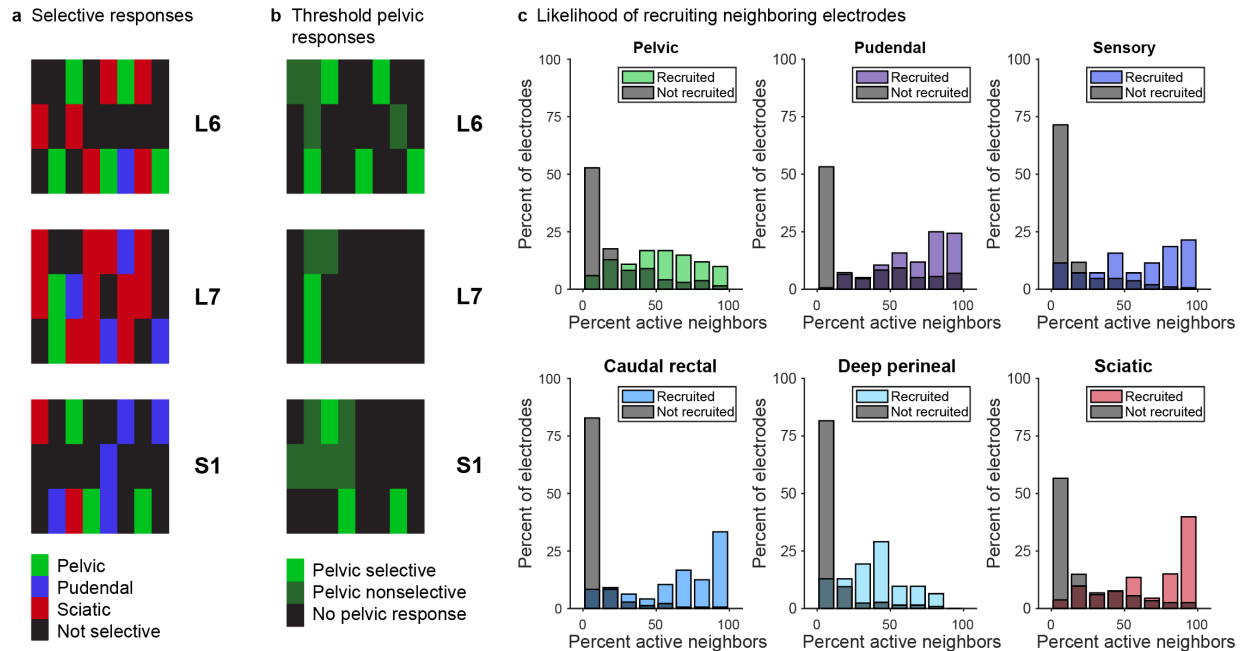
463





464

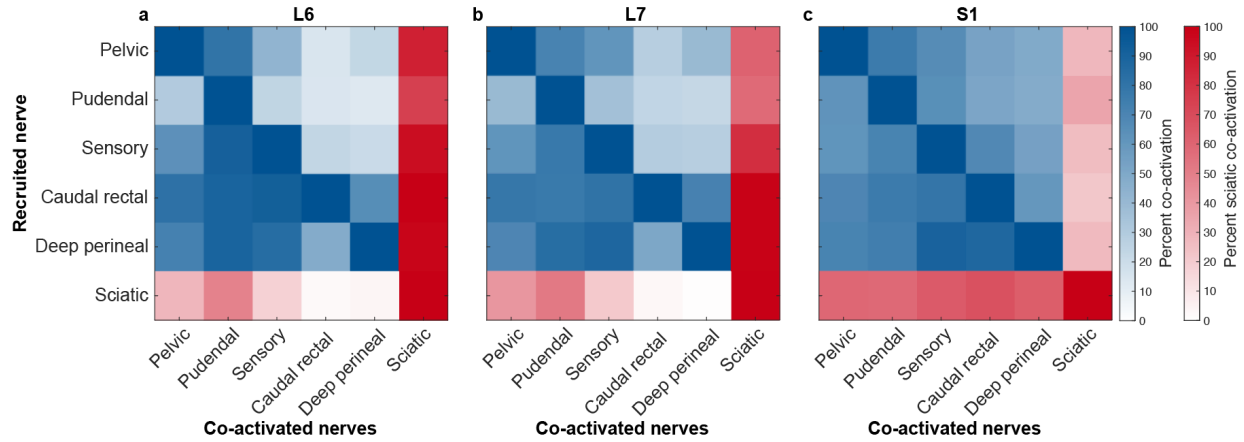
465 **Fig 4. Recruitment of all nerves at threshold at each spinal level.** Selective and nonselective nerve  
466 recruitment at each location at the threshold amplitude. The darkest color (bottom) in each stacked bar is  
467 the median percentage of electrodes that recruited each nerve selectively at the threshold amplitude. The  
468 lighter shade (top) represents the percentage of electrodes that recruited each nerve non-selectively at the  
469 threshold amplitude. Thus, the cumulative total of the bars represents the total recruitment of each nerve at  
470 the threshold amplitude.



471

472 **Figure 5. Spatial arrangement of evoked responses.** a) Selective recruitment for the pelvic nerve,  
 473 pudendal nerve, and sciatic nerve in a representative animal. b) Pelvic nerve recruitment for the same animal  
 474 as panel a, demonstrating that the pelvic nerve was frequently recruited non-selectively on electrodes  
 475 adjacent to selective electrodes. c) When an electrode activated a particular nerve at the threshold amplitude,  
 476 neighboring electrodes were likely to activate that nerve as well (colored bars). The y-axis is normalized to  
 477 the total number of electrodes that activated a specific nerve. When that nerve had not been activated,  
 478 surrounding electrodes were much less likely to activate neighboring electrodes (gray bars). The y-axis for  
 479 the gray bars is normalized to the total number of electrodes that did not activate a specific nerve.

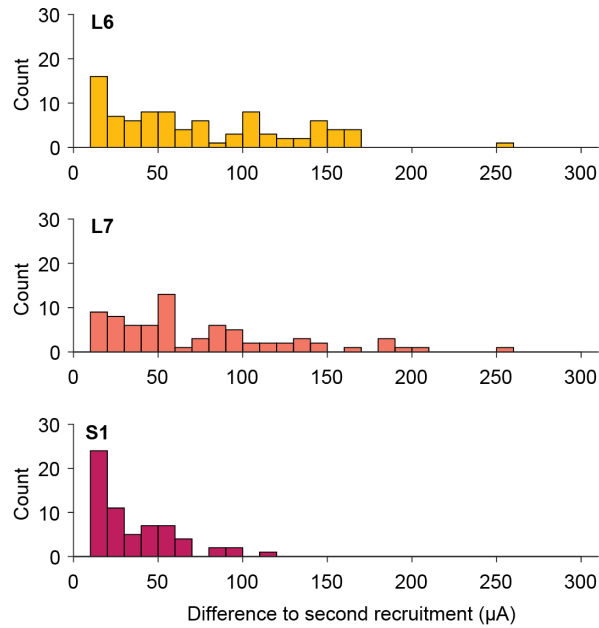
480



481

482 **Figure 6. Coactivation of all nerves.** When a nerve first became active (vertical axis), other nerves are  
483 often co-activated or were recruited at lower amplitudes (horizontal axis). Sciatic comparisons are colored  
484 differently for clarity.

485



486

487 **Figure 7. Distribution of dynamic ranges for each array location.** The dynamic range of each selective  
488 electrode is the amount of additional stimulation current necessary to evoke activity in an additional nerve,  
489 over and above the initial selective response. Many nerves recruited selectively had a dynamic range of less  
490 than 50 µA.

491

492 **References**

493

494 1. Hunskaar, S. *et al.* Epidemiology and natural history of urinary incontinence. *Int Urogynecol J*  
495 *Pel* **11**, 301–19 (2000).

496 2. Hu, T.-W. *et al.* Costs of urinary incontinence and overactive bladder in the United States: a  
497 comparative study. *Urology* **63**, 461–5 (2004).

498 3. Brown, J., McGhan, W. & Chokroverty, S. Comorbidities associated with overactive bladder.  
499 *Am J Manag C* **6**, S574–S579 (2000).

500 4. Simpson, L. A., Eng, J. J., Hsieh, J. T. C. & Wolfe, D. L. The Health and Life Priorities of  
501 Individuals with Spinal Cord Injury: A Systematic Review. *J Neurotraum* **29**, 1548–1555 (2012).

502 5. Benevento, B. T. & Sipski, M. L. Neurogenic bladder, neurogenic bowel, and sexual  
503 dysfunction in people with spinal cord injury. *Phys Ther* **82**, 601–12 (2002).

504 6. Nicolle, L. E. Catheter associated urinary tract infections. *Antimicrob Resist In* **3**, 23 (2014).

505 7. Herrity, A., Williams, C., Angeli, C., Harkema, S. & Hubscher, C. Lumbosacral spinal cord  
506 epidural stimulation improves voiding function after human spinal cord injury. *Sci Rep-UK* **8**,  
507 8688 (2018).

508 8. Gad, P. N. *et al.* Initiation of Bladder Voiding with Epidural Stimulation in Paralyzed, Step  
509 Trained Rats. *PLoS ONE* **9**, e108184 (2014).

510 9. Harkema, S. *et al.* Effect of epidural stimulation of the lumbosacral spinal cord on voluntary  
511 movement, standing, and assisted stepping after motor complete paraplegia: a case study. *Lancet*  
512 **377**, 1938–47 (2011).

513 10. Hubscher, C. H. *et al.* Improvements in bladder, bowel and sexual outcomes following task-  
514 specific locomotor training in human spinal cord injury. *PLoS ONE* **13**, e0190998 (2018).

515 11. Chang, H. H., Yeh, J.-C., Ichiyama, R. M., Rodriguez, L. V. & Havton, L. A. Mapping and  
516 neuromodulation of lower urinary tract function using spinal cord stimulation in female rats. *Exp*  
517 *Neurol* **305**, 26–32 (2018).

518 12. Sayenko, D. G., Angeli, C., Harkema, S. J., Edgerton, V. R. & Gerasimenko, Y. P.  
519 Neuromodulation of evoked muscle potentials induced by epidural spinal-cord stimulation in  
520 paralyzed individuals. *J Neurophysiol* **111**, 1088–1099 (2014).

521 13. Barra, B. *et al.* Electrical Stimulation Of The Cervical Dorsal Roots Enables Functional Arm  
522 And Hand Movements In Monkeys With Cervical Spinal Cord Injury. *Biorxiv*  
523 2020.11.13.379750 (2020) doi:10.1101/2020.11.13.379750.

- 524 14. Capogrosso, M. *et al.* A Computational Model for Epidural Electrical Stimulation of Spinal  
525 Sensorimotor Circuits. *J Neurosci* **33**, 19326–19340 (2013).
- 526 15. Minassian, K. *et al.* Human lumbar cord circuitries can be activated by extrinsic tonic input  
527 to generate locomotor-like activity. *Hum Movement Sci* **26**, 275–295 (2007).
- 528 16. Moraud, E. M. *et al.* Mechanisms Underlying the Neuromodulation of Spinal Circuits for  
529 Correcting Gait and Balance Deficits after Spinal Cord Injury. *Neuron* **89**, 814–828 (2016).
- 530 17. Lavrov, I. *et al.* Facilitation of Stepping with Epidural Stimulation in Spinal Rats: Role of  
531 Sensory Input. *J Neurosci* **28**, 7774–7780 (2008).
- 532 18. Boggs, J. W., Wenzel, B. J., Gustafson, K. J. & Grill, W. M. Frequency-dependent selection  
533 of reflexes by pudendal afferents in the cat. *J Physiol* **577**, 115–126 (2006).
- 534 19. Creed, K. E. & Tulloch, A. G. S. The Effect of Pelvic Nerve Stimulation and Some Drugs on  
535 the Urethra and Bladder of the Dog. *Brit J Urol* **50**, 398–405 (1978).
- 536 20. Bradley, W. & Teague, C. Electrophysiology of pelvic and pudendal nerves in the cat. *Exp*  
537 *Neurol* **35**, 378–393 (1972).
- 538 21. Tai, C., Wang, J., Wang, X., Groat, W. C. de & Roppolo, J. R. Bladder inhibition or voiding  
539 induced by pudendal nerve stimulation in chronic spinal cord injured cats. *Neurourol Urodynam*  
540 **26**, 570–577 (2007).
- 541 22. Yoo, P. B., Woock, J. P. & Grill, W. M. Bladder activation by selective stimulation of  
542 pudendal nerve afferents in the cat. *Exp Neurol* **212**, 218–225 (2008).
- 543 23. Woock, J. P., Yoo, P. B. & Grill, W. M. Activation and inhibition of the micturition reflex by  
544 penile afferents in the cat. *Am J Physiol Regul Integ Comp Physiol* **294**, R1880–R1889 (2008).
- 545 24. McGee, M. J. & Grill, W. M. Selective co-stimulation of pudendal afferents enhances  
546 bladder activation and improves voiding efficiency. *Neurourology and Urodynamics* **33**, 1272–  
547 1278 (2014).
- 548 25. Woock, J. P., Yoo, P. B. & Grill, W. M. Mechanisms of reflex bladder activation by  
549 pudendal afferents. *Am J Physiol Regul Integ Comp Physiol* **300**, R398–R407 (2011).
- 550 26. Mashni, J. W. & Peters, K. M. Potential Use of Pudendal Nerve Stimulation for Voiding  
551 Dysfunction. *Curr Bladder Dysfunct Rep* **5**, 177–182 (2010).
- 552 27. Martens, F. M. J., Heesakkers, J. P. F. A. & Rijkhoff, N. J. M. Surgical Access for Electrical  
553 Stimulation of the Pudendal and Dorsal Genital Nerves in the Overactive Bladder: A Review. *J*  
554 *Urology* **186**, 798–804 (2011).

- 555 28. Gustafson, K. J. *et al.* Fascicular anatomy and surgical access of the human pudendal nerve.  
556 *World J Urol* **23**, 411–418 (2005).
- 557 29. O’Bichere, A., Green, C. & Phillips, R. K. S. New, simple approach for maximal pudendal  
558 nerve exposure. *Dis Colon Rectum* **43**, 956–960 (2000).
- 559 30. Lee, J. F., Maurer, V. M. & Block, G. E. Anatomic Relations of Pelvic Autonomic Nerves to  
560 Pelvic Operations. *Arch Surg-Chicago* **107**, 324 (1973).
- 561 31. Guiho, T. *et al.* Functional Selectivity of Lumbosacral Stimulation: Methodological  
562 Approach and Pilot Study to Assess Visceral Function in Pigs. *IEEE T Neur Sys Reh* **26**, 2165–  
563 2178 (2018).
- 564 32. Bradley, K. The technology: the anatomy of a spinal cord and nerve root stimulator: the lead  
565 and the power source. *Pain Med* **7**, S27–S34 (2006).
- 566 33. Burgio, K. L., Engel, B. T., Quilter, R. E. & Arena, V. C. The relationship between external  
567 anal and external urethral sphincter activity in continent women. *Neurourol Urodynam* **10**, 555–  
568 562 (1991).
- 569 34. Brindley, G. S., Polkey, C. E., Rushton, D. N. & Cardozo, L. Sacral anterior root stimulators  
570 for bladder control in paraplegia: the first 50 cases. *J Neurology Neurosurg Psychiatry* **49**, 1104–  
571 1114 (1986).
- 572 35. Martens, F. M. J. *et al.* Quality of life in complete spinal cord injury patients with a Brindley  
573 bladder stimulator compared to a matched control group. *Neurourol Urodynam* **30**, 551–555  
574 (2011).
- 575 36. Groat, W. C. de & Ryall, R. W. Reflexes to sacral parasympathetic neurones concerned with  
576 micturition in the cat. *J Physiology* **200**, 87–108 (1969).
- 577 37. Groat, W. C. de & Theobald, R. J. Reflex activation of sympathetic pathways to vesical  
578 smooth muscle and parasympathetic ganglia by electrical stimulation of vesical afferents. *J*  
579 *Physiology* **259**, 223–237 (1976).
- 580 38. Woźniak, W. & Skowrońska, U. Comparative anatomy of pelvic plexus in cat, dog, rabbit,  
581 macaque and man. *Anat Anzeiger* **120**, 457–73 (1967).
- 582 39. Susset, J. G. & Boctor, Z. N. Implantable Electrical Vesical Stimulator : Clinical Experience.  
583 *J Urology* **98**, 673–678 (1967).
- 584 40. Gad, P. *et al.* Development of a multi-electrode array for spinal cord epidural stimulation to  
585 facilitate stepping and standing after a complete spinal cord injury in adult rats. *J Neuroeng*  
586 *Rehabil* **10**, 2 (2013).

- 587 41. Greiner, N. *et al.* Recruitment of Upper-Limb Motoneurons with Epidural Electrical  
588 Stimulation of the Primate Cervical Spinal Cord. *Biorxiv* 2020.02.17.952796 (2020)  
589 doi:10.1101/2020.02.17.952796.
- 590 42. Barra, B. *et al.* Selective Recruitment of Arm Motoneurons in Nonhuman Primates Using  
591 Epidural Electrical Stimulation of the Cervical Spinal Cord. in *IEEE Engineering in Medicine  
592 and Biology Society* 1424–1427 (2018). doi:10.1109/EMBC.2018.8512554.
- 593 43. Pettigrew, R. I. *et al.* Epidural Spinal Stimulation to Improve Bladder, Bowel, and Sexual  
594 Function in Individuals With Spinal Cord Injuries: A Framework for Clinical Research. *IEEE T  
595 Bio-Med Eng* **64**, 253–262 (2017).
- 596 44. Huang, R. *et al.* Modulation of respiratory output by cervical epidural stimulation in the  
597 anesthetized mouse. *J Appl Physiol* **121**, 1272–1281 (2016).
- 598 45. Harkema, S. J. *et al.* Normalization of Blood Pressure With Spinal Cord Epidural Stimulation  
599 After Severe Spinal Cord Injury. *Front Hum Neurosci* **12**, 83 (2018).
- 600 46. Kumar, K. *et al.* Spinal Cord Stimulation: Placement of Surgical Leads via Laminotomy –  
601 Techniques and Benefits. in *Neuromodulation* 1005–1011 (Elsevier, 2009). doi:10.1016/b978-0-  
602 12-374248-3.00087-2.
- 603 47. Olin, J. C., Kidd, D. H. & North, R. B. Postural Changes in Spinal Cord Stimulation  
604 Perceptual Thresholds. *Neuromodulation Technology Neural Interface* **1**, 171–175 (1998).
- 605 48. Villavicencio, A. T., Leveque, J.-C., Rubin, L., Bulsara, K. & Gorecki, J. P. Laminectomy  
606 versus Percutaneous Electrode Placement for Spinal Cord Stimulation. *Neurosurgery* **46**, 399–  
607 406 (2000).
- 608 49. Deer, T. R. *et al.* The appropriate use of neurostimulation of the spinal cord and peripheral  
609 nervous system for the treatment of chronic pain and ischemic diseases: the Neuromodulation  
610 Appropriateness Consensus Committee. *Neuromodulation J Int Neuromodulation Soc* **17**, 515–  
611 50; discussion 550 (2014).
- 612 50. Vanderhorst, V. & Holstege, G. Organization of lumbosacral motoneuronal cell groups  
613 innervating hindlimb, pelvic floor, and axial muscles in the cat. *J Comp Neurol* (1997).
- 614 51. Kawatani, M., Nagel, J. & Groat, W. C. D. Identification of neuropeptides in pelvic and  
615 pudendal nerve afferent pathways to the sacral spinal cord of the cat. *J Comp Neurol* **249**, 117–  
616 132 (1986).
- 617 52. Groat, W. C. de & Yoshimura, N. Afferent nerve regulation of bladder function in health and  
618 disease. *Handbook of experimental pharmacology* 91–138 (2009) doi:10.1007/978-3-540-79090-  
619 7\_4.



- 620 53. Groat, W. de, Griffiths, D. & Yoshimura, N. Neural control of the lower urinary tract.  
621 *Comprehensive Physiology* **5**, 327–396 (2015).
- 622 54. Martin, W. D., Fletcher, T. F. & Bradley, W. E. Innervation of feline perineal musculature.  
623 *The Anatomical Record* **180**, 15–29 (1974).
- 624 55. Thor, K., Morgan, C., Nadelhaft, I., Houston, M. & Groat, W. de. Organization of afferent  
625 and efferent pathways in the pudendal nerve of the female cat. *J Comp Neurol* **288**, 263–279  
626 (1989).
- 627 56. Morgan, C., Nadelhaft, I. & Groat, W. C. de. The distribution of visceral primary afferents  
628 from the pelvic nerve to Lissauer’s tract and the spinal gray matter and its relationship to the  
629 sacral parasympathetic nucleus. *J Comp Neurol* **201**, 415–440 (1981).
- 630 57. Powell, C. R. Troubleshooting Interstim Sacral Neuromodulation Generators to Recover  
631 Function. *Curr Urol Rep* **19**, 86 (2018).
- 632 58. Brindley, G. S. & Rushton, D. N. Long-term follow-up of patients with sacral anterior root  
633 stimulator implants. *Spinal Cord* **28**, 469–475 (1990).
- 634 59. Tai, C. *et al.* Prolonged poststimulation inhibition of bladder activity induced by tibial nerve  
635 stimulation in cats. *Am J Physiol Renal Physiol* **300**, F385–F392 (2011).
- 636 60. Holsheimer, J., Struijk, J. & Tas, N. Effects of electrode geometry and combination on nerve  
637 fibre selectivity in spinal cord stimulation. *Med Biol Eng Comput* **33**, 676–682 (1995).
- 638 61. Chandrasekaran, S. *et al.* Sensory restoration by epidural stimulation of the lateral spinal cord  
639 in upper-limb amputees. *Elife* **9**, e54349 (2020).
- 640 62. Kumar, K., Toth, C., Nath, R. & Laing, P. Epidural spinal cord stimulation for treatment of  
641 chronic pain—some predictors of success. A 15-year experience. *Surg Neurol* **50**, 110–121  
642 (1998).
- 643 63. Grill, W. & Mortimer, J. Stimulus waveforms for selective neural stimulation. *IEEE Eng*  
644 *Med Biol* 375–385 (1995).
- 645 64. McIntyre, C. C. & Grill, W. M. Selective Microstimulation of Central Nervous System  
646 Neurons. *Annals of Biomedical Engineering* **28**, 219–233 (2000).
- 647 65. Qing, K. Y., Ward, M. P. & Irazoqui, P. P. Burst-Modulated Waveforms Optimize Electrical  
648 Stimuli for Charge Efficiency and Fiber Selectivity. *IEEE T Neur Sys Reh* **23**, 936–945 (2015).
- 649 66. Squair, J. W. *et al.* Neuroprosthetic baroreflex controls haemodynamics after spinal cord  
650 injury. *Nature* **590**, 308–314 (2021).

- 651 67. Ayers, C. A., Fisher, L. E., Gaunt, R. A. & Weber, D. J. Microstimulation of the lumbar  
652 DRG recruits primary afferent neurons in localized regions of lower limb. *J Neurophysiol* **116**,  
653 51–60 (2016).
- 654 68. Nanivadekar, A., Ayers, C. A., Gaunt, R. A., Weber, D. & Fisher, L. E. Selectivity of  
655 afferent microstimulation at the DRG using epineural and penetrating electrode arrays. *J Neural*  
656 *Eng* (2019) doi:10.1088/1741-2552/ab4a24.
- 657 69. Gaunt, R. *et al.* Lower urinary track nerve responses to high-density epidural spinal cord  
658 stimulation ML-RNEL 4908 Bladder SCS. *Blackfynn Discover* (2020) doi:10.26275/iami-zirb.
- 659